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THE GROWTH OF HYPOPHYSECTOMIZED FEMALE RATS FOLLOWING CHRONIC TREATMENT WITH PURE PITUITARY GROWTH HORMONE[1]

I. General Growth and Organ Changes

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(Received for publication April 27, 1949)

It has been known for a considerable time that the growth of hypophysectomized animals may be reinvoked by the administration of crude anterior hypophyseal extracts. Injection of crude alkaline extracts has led to continuous growth for several months. During the period when continued efforts were made by us and others to purify the growth hormone, the injection of partially purified growth extracts resulted in the prompt resumption of growth in hypophysectomized animals, but it was found that within from 60 to 100 days, growth was slowed and finally ceased (Evans, 1935). Several explanations were advanced for this failure in the continuity of the growth response. Collip and his co-workers (Collip, 1934) were inclined to look upon the phenomenon as evidence for the gradual formation of a specific inhibitory substance which might be designated the anti-growth hormone. On the other hand, investigators naturally bore in mind the certainty that hypophysectomized animals suffered from other deficiencies than those involved in the elimination of the growth hormone. Such a line of thought led us and others to inquire into the role in growth of other hypophyseal hormones, the so-called "target organ hormones," or of the hormones of the so-called target organs themselves, and into the possible disturbance in growth induced in hypophysectomized rats by certain metabolic changes. It was for such reasons that attempts were made to discover whether the conveyance of cortin would reinstate growth in hypophysectomized rats

[1]Aided by grants from the American Cancer Society, Inc., New York City; The United States Public Health Service (RG-409), Washington, D. C.; The Rockefeller Foundation, New York City and the Research Board of the University of California, Berkeley.

whose growth had slowed or ceased under the constant administration of a previously satisfactory growth hormone. Similar inquiries had also prompted the administration of thyroxin or of parenteral glucose. None of these efforts led to conclusive deductions. But, in the course of these studies, it was found that animals whose growth had ceased under the continuous administration of partially purified growth extracts might suddenly resume growth when crude extracts were administered (Evans, Pencharz and Simpson, 1935). Support was hereby given the concept that for the continued growth of hypophysectomized animals, some other substance or substances than the growth hormone itself was required. Progress in the purification of the growth hormone was not impeded by these disclosures. Animals which had recently been hypophysectomized could always be made to grow rapidly for ten, twenty, or thirty days by the administration of any potent, partially purified growth extract and therefore the progress of purification could be assessed adequately. As soon as the growth hormone was obtained as a pure protein (Li, Evans and Simpson, 1945) the effect of its continued administration was retested in normal and hypophysectomized rats. We have already reported the experimental production of gigantism by the treatment of normal animals with the pure growth hormone for a period of 14 months (Evans, Simpson and Li, 1948). The present paper deals with the results secured by the continuous treatment of young hypophysectomized rats for a corresponding period, and it may be said at once that the animals continued to grow throughout the injection period, 437 days.

Conditions of Experiment. Female rats 26 to 28 days of age were hypophysectomized and 12 days after the operation the daily intraperitoneal administration of growth hormone was begun. The dosage was given once daily, 6 days a week, for a period of 437 days. The animals were sacrificed when 476 days of age. The preparation of the pure anterior hypophyseal growth hormone employed contained 50 growth units per milligram of dry weight.[2] The initial daily dose of growth hormone was 0.1 mg.; this was gradually increased to

[2]The unit is defined as the amount necessary to produce a gain of 10 grams in the total body weight when injected once daily for ten days in hypophysectomized female rats, 26 to 28 days of age, the treatment being inaugurated 12 to 14 days after the operation.

0.4 mg.[3] The rats had continuous access to an abundance of a dry food (Diet XIV[4]), known to be adequate in all respects for satisfactory growth. The diet was supplemented daily by a wet mash made from Diet I.[5] Fresh lettuce was fed twice or thrice weekly. The animals were kept under clean conditions and a practically uniform temperature (72° F.), and so placed as to be protected from air currents. They were weighed at 5-day intervals. The tail length (anus to tail tip) was measured at monthly intervals as a general index of skeletal growth in linear dimensions.

Fifteen treated and seventeen untreated rats were originally placed on the experiment, but only 5 treated and 5 untreated rats survived for the fourteen month's period.

At the end of the experiment, the animals were anesthetized with amytal in order to permit samples of the blood, liver, and striated musculature to be taken for chemical analyses. The sella turcica was checked for the possible presence of pituitary fragments. The organs were weighed carefully before being fixed for histological analysis; the skeleton was also fixed for histological exploration.

The growth in body weight and in dimensions is apparent in Figures 1 and 2 showing photographs of hypophysectomized controls and

TABLE I

BODY WEIGHT AND LENGTH OF HYPOPHYSECTOMIZED FEMALE RATS INJECTED WITH PITUITARY GROWTH HORMONE FOR 437 DAYS

Group	No. of Rats	Body Weight			Length
		Onset	Autopsy	Gain	At Autopsy
		gm.	gm.	gm.	cm.
Growth Hormone	5	69 ± 0.5[1]	410 ± 27.6	341 ± 27.5	39.2 ± 0.72
Control	5	67 ± 1.6	104 ± 6.4	37 ± 6.7	27.9 ± 0.33

[1]The figures preceded by ± are the Standard Errors.

[3]0.1 mg. was given daily for 111 doses; 0.2 mg. was given daily for 270 doses; whereupon 0.4 mg. was given daily for 22 doses.

[4]Diet XIV: ground whole wheat, 68.5%; casein, technical, 5.0%; fish oil, 5.0%; alfalfa leaf meal, 10.0%; fish meal, 10.0%; sodium chloride, 1.5%. Approximate composition: 19.3% protein, 56.7% carbohydrate and 6.5% fat.

[5]Diet I: ground whole wheat, 67.5%; casein, technical, 15.0%; whole milk powder, 10.0%; hydrogenated vegetable oil, 5.25%; calcium carbonate, 1.5%; sodium chloride, iodized, 0.75%. 16 cc. Sardilene (vitamin A-D concentrate) is added per kg. diet. This diet is ordinarily used only during gestation and lactation. Approximate composition is 24.3% protein, 54.8% carbohydrate and 11% fat.

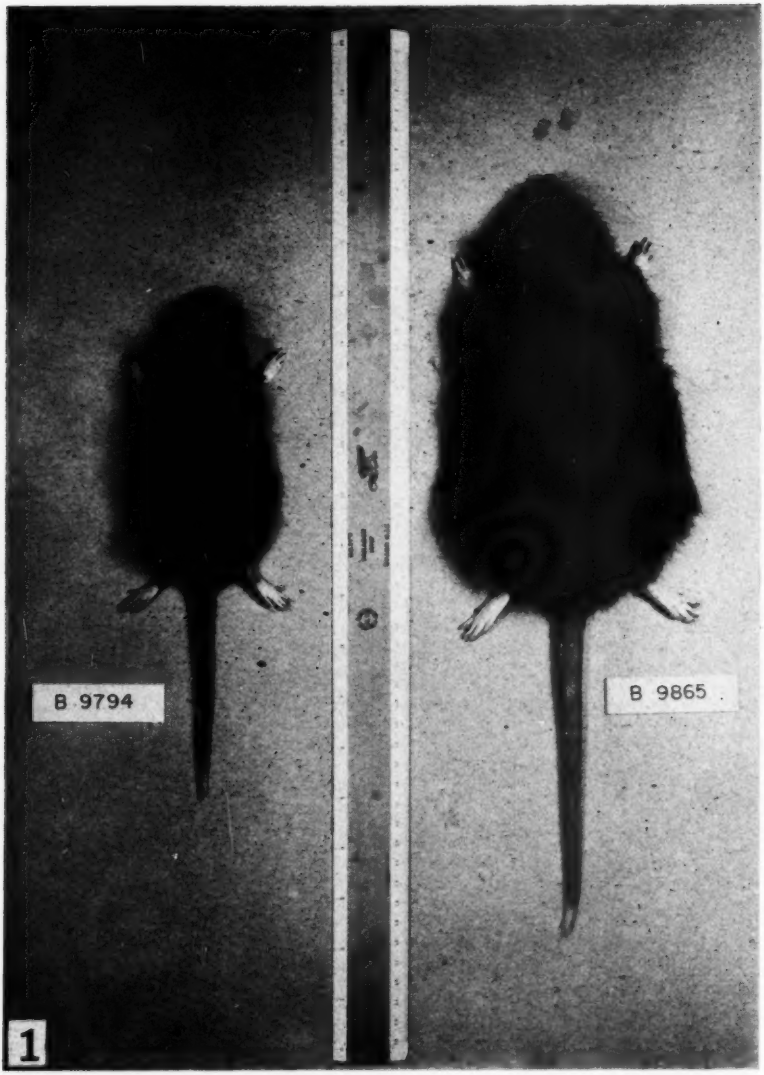


FIGURE 1

HYPOPHYSECTOMIZED FEMALE RATS PHOTOGRAPHED UNDER ANESTHESIA, 449 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE

Left: Untreated control.

Right: Rat injected with growth hormone for 437 days beginning 12 days after the operation.

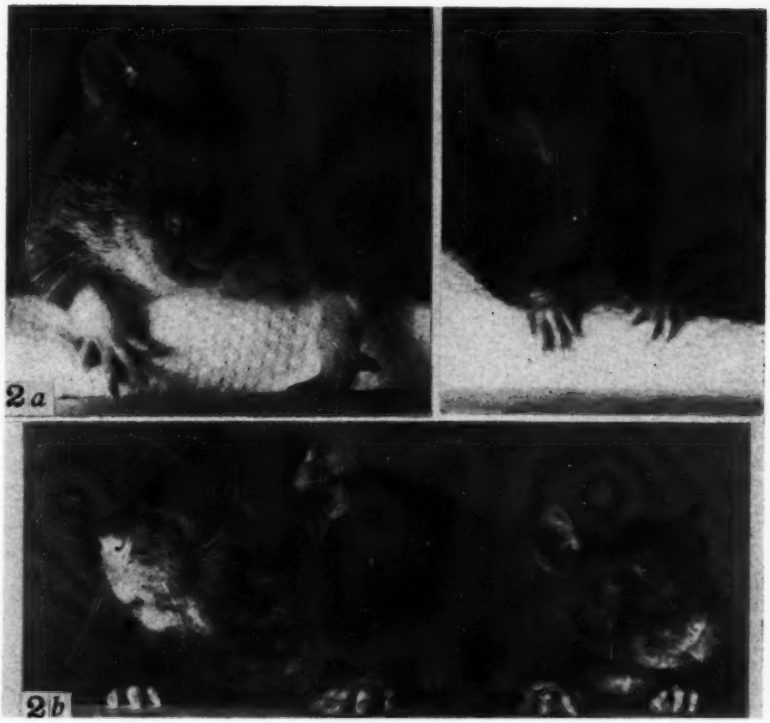


FIGURE 2
HYPOPHYSECTOMIZED FEMALE RATS PHOTOGRAPHED 449 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE
The growth hormone injected rat is on the left in both a and b.

chronically injected rats. The growth is further analyzed in Table I. It will be seen from the graph (Fig. 3) that growth continued in the injected rats to the end of the experimental period although not at the initial rate, whereas virtually no increase in body weight occurred in the hypophysectomized controls. At autopsy, the injected animals were approximately four times the weight of the controls and their skeletal dimensions were almost one and one-half times that of the controls.

The injected rats were sluggish, although their muscle tone was

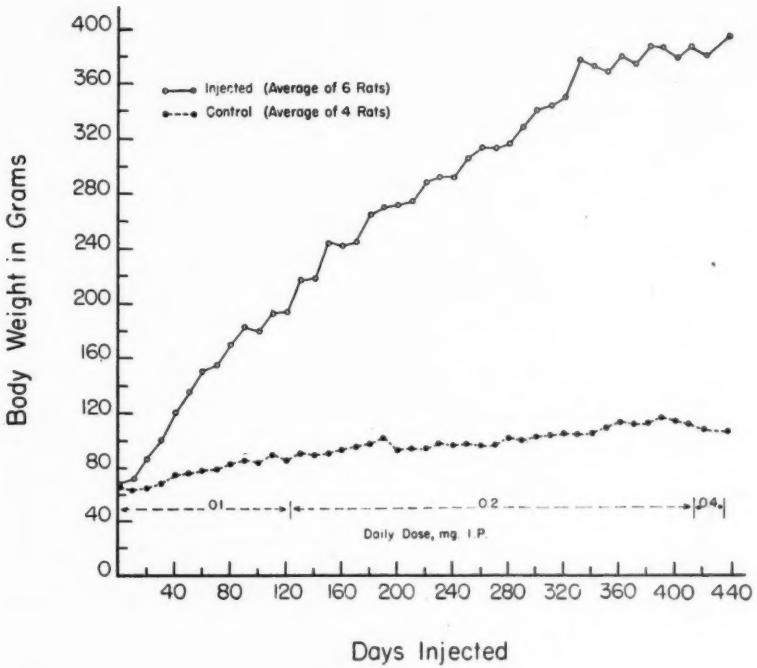


FIGURE 3

AVERAGE BODY WEIGHT CURVE OF GROWTH HORMONE INJECTED HYPOPHYSECTOMIZED FEMALE RATS COMPARED WITH THAT OF UNINJECTED CONTROLS, DURING A 437 DAY EXPERIMENTAL PERIOD, BEGINNING 12 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE

good. Their noses and paws were pinker than in controls. The hair was long, soft and lusterless; the guard hairs were poorly developed. The vibrissae were coarse, and were irregular in length and frequently curved and broken (see Fig. 2b). They seemed less vibratile than in the normal. The skin was redundant—large folds could be plucked up. Abnormalities of the pelvic girdle and the thoracic cage were noted ("pigeon breast", "shelf-like" pelvis).[6] The wrists and ankles were thick, as were the digits. The paws were, however, not elongated as much as would have been anticipated from the general body

[6]The changes in the thoracic cage and pelvis will be described in the subsequent papers devoted to skeletal change.

growth. The head was not long and slender as in normal animals but thick laterally and dorso-ventrally. The nose was coarse, the cheeks could be properly called "jowls" (the appearance of the head was in part due to the skeletal changes but also due to muscle hypertrophy). The eyes were less prominent than normal. The phallus was enlarged and there was a fullness between anus and vaginal orifice suggesting a scrotum. The nipples were long. In contrast with the injected animals, uninjected hypophysectomized controls had poor muscle tone; the ears and nose were pale. The eyes were prominent.

At autopsy the injected animals showed large amounts of subcutaneous and intraperitoneal fat. Muscular hypertrophy was evident. There was complete absence of neoplasms in both the injected and uninjected hypophysectomized rats.[7] The adrenals were observed to be larger than in the control animals and it was predicted that this enlargement would be found to be due to enlargement of the medulla, as the adrenals were mottled, indicating that the cortex was as thin as in the hypophysectomized controls. The ovaries and uteri were atrophic. In spite of this atrophy of the ovaries, the vaginas of the injected animals were open, whereas in controls all vaginal membranes were intact. The thymus, though reduced in size, was semi-translucent and glistening as in the normal, whereas only a tag of

TABLE II

WEIGHTS OF VISCERA OF HYPOPHYSECTOMIZED FEMALE RATS INJECTED WITH PITUITARY GROWTH HORMONE FOR 437 DAYS

Group	Body Weight	Liver	Spleen	Kidneys	Stomach	Intestine	Heart	Brain	Eyeball
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Growth Hormone	410	13.67 ±0.43	0.91 ±0.06	1.50 ±0.06	1.83 ±0.83	14.19 ±0.70	1.07 ±0.06	1.98 ±0.04	0.177 ±0.003
	% B.W.	3.33	0.22	0.36	0.45	3.45	0.26	0.48	0.04
Control	104	3.42 ±0.28	0.20 ±0.02	0.66 ±0.02	0.72 ±0.04	7.43 ±0.32	0.40 ±0.05	1.60 ±0.05	0.160 ±0.002
	% B.W.	3.28	0.19	0.63	0.69	7.14	0.38	1.54	0.15

[7]This was in contrast to the frequent occurrence and multiple types of tumors encountered on administration of growth hormone for the same period to animals possessing a pituitary. It should be noted, however, that the maximum daily dose injected into the hypophysectomized rats of this series was 0.4 mg. whereas this was the initial dose in the intact animals; further, the dose given intact rats was increased to 2 mg. daily before the end of the injection period.

tissue scarcely recognizable as thymus remained in the hypophysectomized controls.

Table II shows that the abdominal and thoracic viscera (liver, spleen, kidneys, stomach, intestine, heart) were all remarkably increased in weight. It will be noted that the weights of liver and spleen were increased to four times the weight of these organs in the control animals. The intestine and heart were not as markedly increased; they were only about twice to three times the size of these organs in controls. The weights of the brain and eyeball are also included in this table, and it will be noted that there was practically no increase in the size of these organs.[8] It will be noted that the weight of the liver retained the same proportion to the body weight as in the hypophysectomized controls; the stomach, intestine, heart, and kidney did not quite maintain the same proportionality.[9]

Table III shows the weights of the endocrine and reproductive organs, uterus, preputials, adrenals, thyroid and thymus. Although as the table shows these organs had increased somewhat in weight, their proportionality to body weight was greatly decreased.

The ovaries were not weighed as they were so small that it was considered advisable to fix them directly for histological examination.

The ovaries of the controls were found to consist chiefly of masses of deficient interstitial cells; there were a few small follicles, not sufficiently developed to contain an antrum. The ovaries of the injected animals showed neither follicular development nor interstitial cell repair (Figs. 4 and 5). The Fallopian tube was larger in the

[8]This is not surprising when one recalls that the period of most rapid growth of the nervous tissue has passed at the time of hypophysectomy and onset of the experiment.

[9]The relation of some of these visceral weights to those of normal female rats may be seen from the following figures. The normal females were of greater chronological age than the animals used in this series but still were 100 gm. less in body weight. They may be identified as the controls to the series previously reported in which growth hormone was injected chronically into intact rats.

Type of Rat		Body Weight	Liver	Kidneys	Stomach	Intestine	Heart
		gm.	gm.	gm.	gm.	gm.	gm.
Injected							
Hypophysectomized	Av. 7	410	13.67	1.50	1.83	14.19	1.07
	% BW		3.33	0.36	0.45	3.45	0.26
Normal	Av. 5	301	13.07	2.10	1.50	8.84	0.99
Control	% BW		4.34	0.69	0.40	2.93	0.33

TABLE III

WEIGHTS OF REPRODUCTIVE AND ENDOCRINE ORGANS OF HYPOPHYSECTOMIZED FEMALE RATS INJECTED WITH PITUITARY GROWTH HORMONE FOR 437 DAYS*

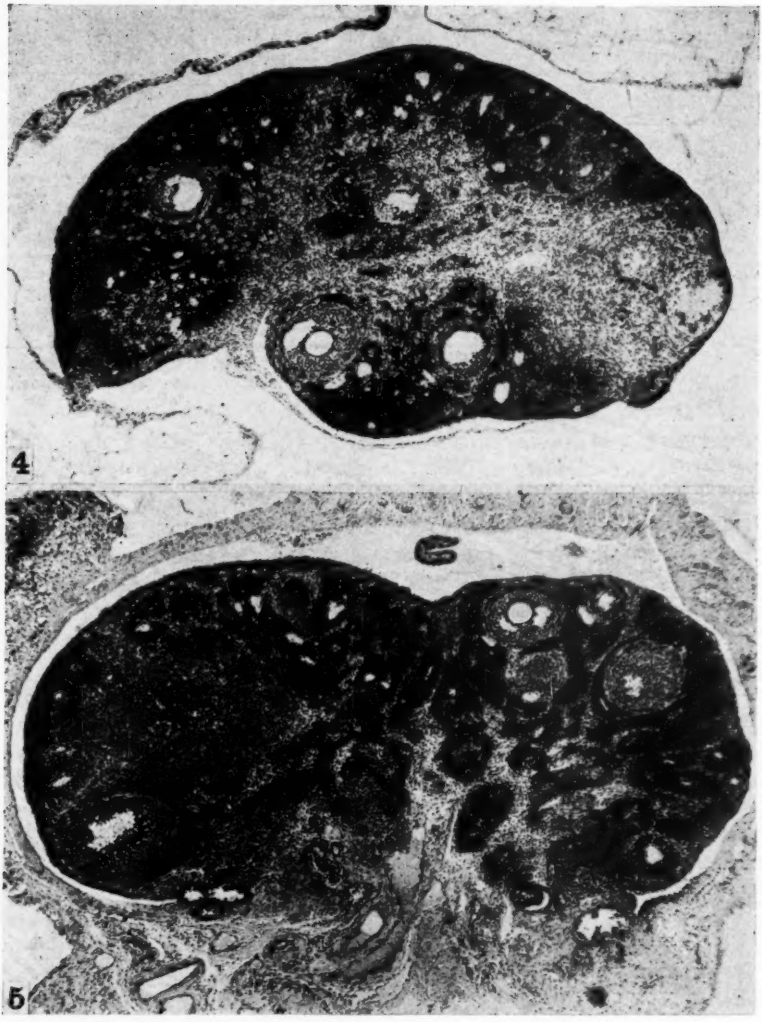
Group	Body Weight	Uterus	Preputials	Thyroid	Adrenals	Thymus
	gm.	mg.	mg.	mg.	mg.	mg.
Growth Hormone	410	66 ± 4.43	52 ± 4.40	19 ± 2.00	24 ± 1.36	87 ±15.28
	mg./100 gm. B.W.	16.0	12.7	4.5	5.9	21.2
Control	104	22 ± 2.28	19 ± 4.25	10 ± 0.45	13 ± 2.21	24 ± 2.85
	mg./100 gm. B.W.	21.1	18.2	9.6	12.5	23.0

*The weights of these organs in normal adult female rats of the Long-Evans strain may be given for comparative purposes: uterus, 1073 mg.; preputials, 75 mg.; thyroid, 44 mg.; adrenals, 72 mg.

injected than in the controls. The uterus not only weighed more but was greater in diameter. The endometrial stroma stained less deeply with hematoxylin, due to the fact that the nuclei were somewhat less pyknotic. The uterine glands were somewhat larger and more numerous. The epithelium of the uterus was not distinctly higher than in the controls. The mammary gland (Figs. 6 and 7) showed somewhat more development. The ducts were coarser and a few alveolar buds were present, though none were seen in the controls. The area covered by the mammary glands was not increased.

The thyroids were, as reported, larger than in the control, though here also it was difficult to attribute the increase to specific structural elements. The epithelium was still extremely flat, although the nuclei took a slightly lighter stain. To some extent the increase in the size of the gland was due to the accumulation of colloid (vesicles of the thyroid of one anima! were large enough to be seen at autopsy with the binocular at a magnification of ten fold). The colloid showed no more vacuolation than that in controls (Figs. 8 and 9). The parathyroids were quite large in both the injected and control animals and there was an increased amount of fibrous tissue; both characteristics were perhaps attributable to the age of the animals.[10]

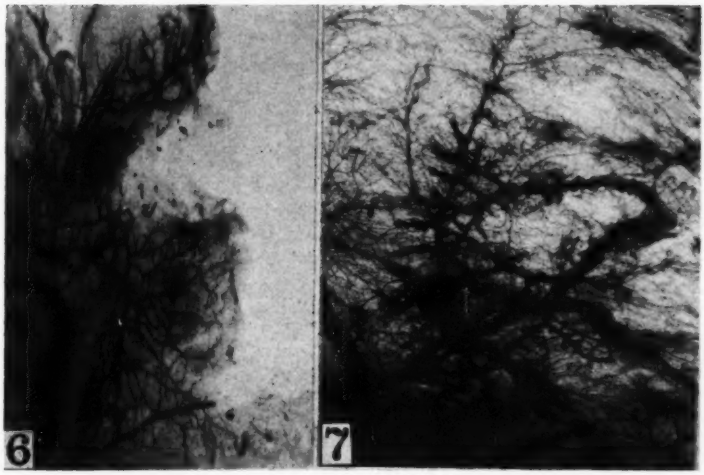
[10]An adenoma of the parathyroid was observed in one of the injected animals, but careful study of the roentgenogram of the entire skeleton of this animal did not disclose any abnormalities. Metaplasia of the thyroid occurred as frequently in the controls as in the experimental animals and was probably a phenomenon associated with their age.



OVARIES OF HYPOPHYSECTOMIZED RATS, 449 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE
H. and E. × 52

FIGURE 4
UNTREATED CONTROL

FIGURE 5
GROWTH HORMONE INJECTIONS FOR 437 DAYS



MAMMARY GLANDS OF HYPOPHYSECTOMIZED RATS, 449 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE
Carmine stain, photographed by transmitted light, × 5.

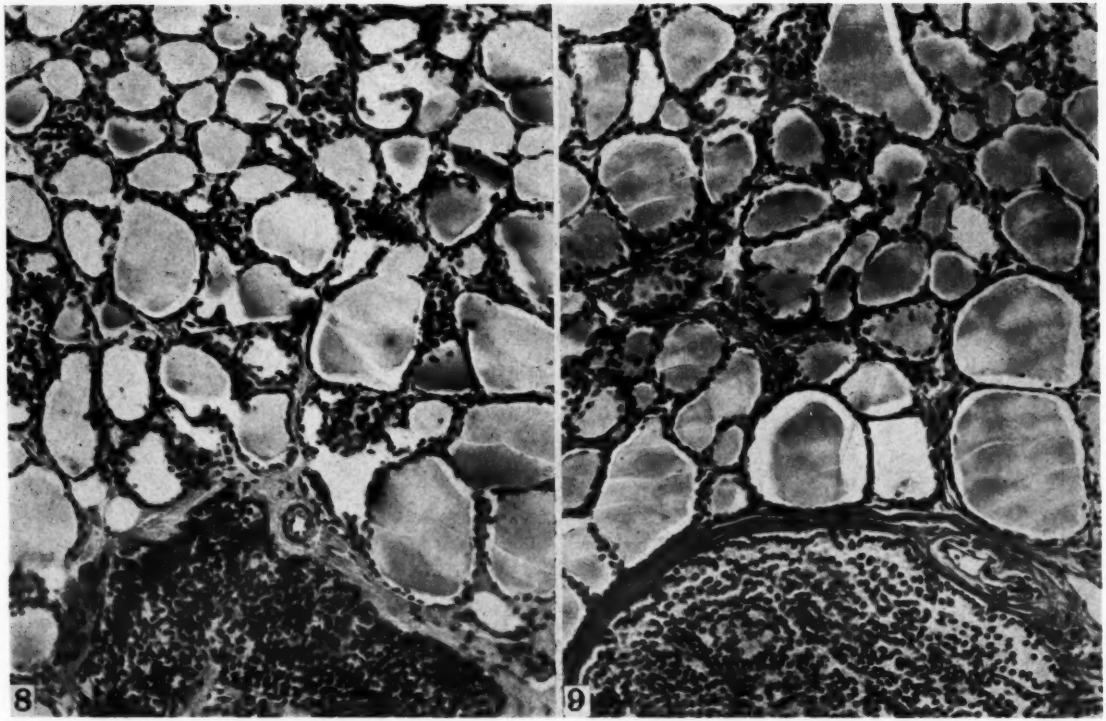
FIGURE 6
UNTREATED CONTROL

FIGURE 7
GROWTH HORMONE INJECTIONS FOR 437 DAYS

As predicted from autopsy observations the adrenal cortex was only slightly increased in width (Figs. 10 and 11). The cortical cells were also slightly larger in the experimental than in the control animals. The lipid distribution as shown by Sudan Orange was the same as in the controls. What increase in size of the adrenal occurred, appeared to be due to enlargement of the medulla rather than of the cortex.[11] (The ratio of the diameter of the medulla to that of the whole adrenal was raised from 0.25 in the controls to 0.36 in injected rats.) The medullary cells were larger in the experimental than in the control rats and mitoses in them were not infrequent (Figs. 12 and 13).

In the kidney the increase in weight was attributable to increase in size of both the tubules and of the glomeruli, which were measurably larger (Figs. 14 and 15).

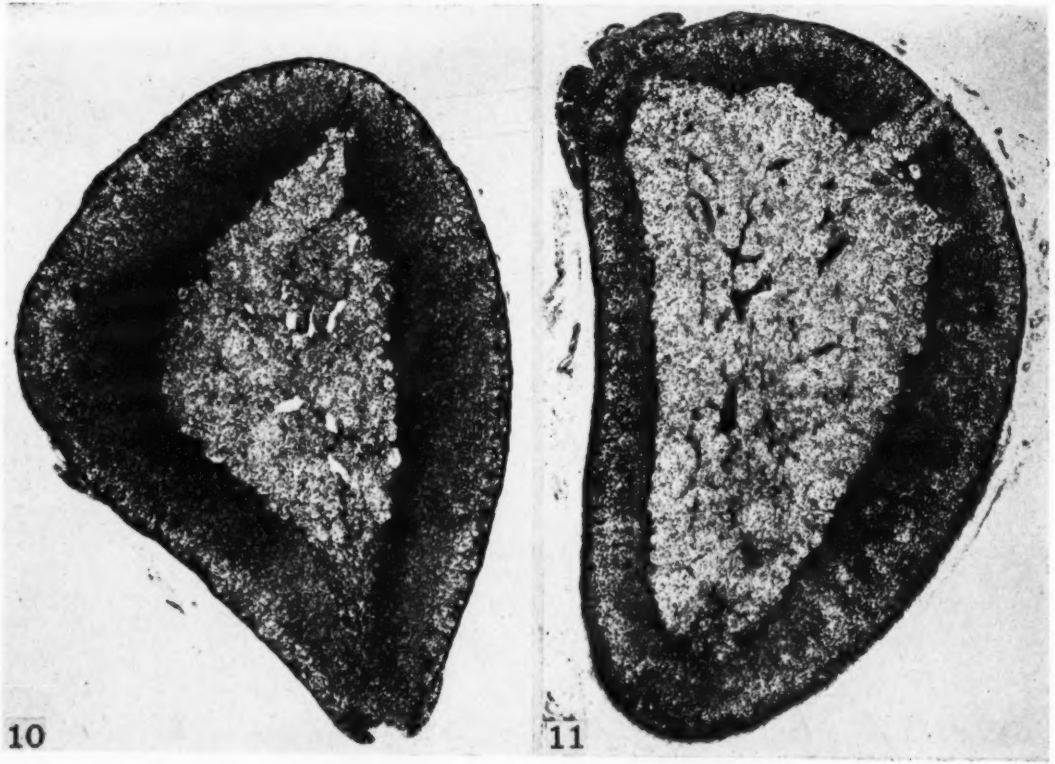
[11]Intact rats injected chronically with growth hormone also showed enlargement of the medulla (Evans, Simpson and Li, 1948).



THYROID GLANDS OF HYPOPHYSECTOMIZED FEMALE RATS, 449 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE. H. and E. × 189.

FIGURE 8
UNTREATED CONTROL

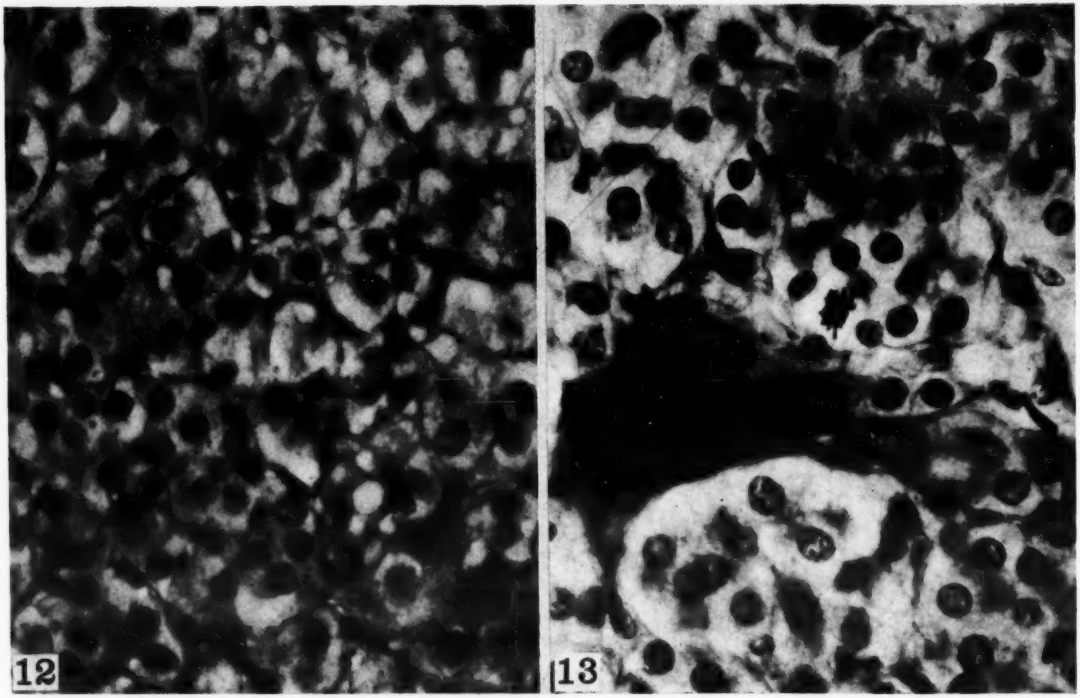
FIGURE 9
GROWTH HORMONE INJECTIONS FOR 437 DAYS



ADRENAL GLANDS OF HYPOPHYSECTOMIZED FEMALE RATS, 449 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE. H. and E. × 84.

FIGURE 10
UNTREATED CONTROL

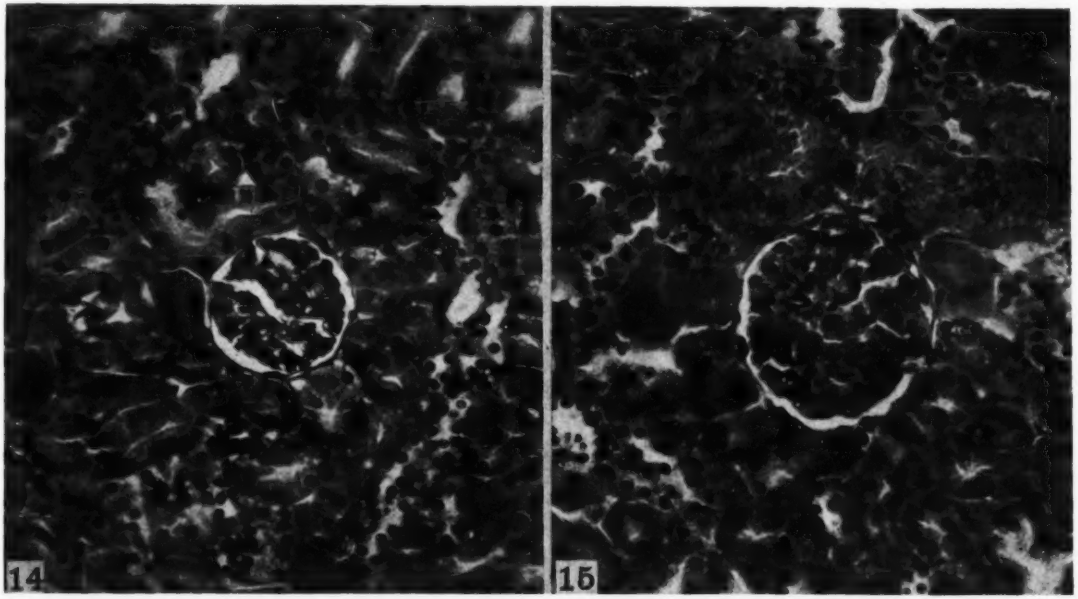
FIGURE 11
GROWTH HORMONE INJECTIONS FOR 437 DAYS



ADRENAL MEDULLA OF HYPOPHYSECTOMIZED FEMALE RATS 449 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE. H. and E. × 640.

FIGURE 12
UNTREATED CONTROL

FIGURE 13
GROWTH HORMONE INJECTIONS FOR 437 DAYS
Note mitotic figure



KIDNEYS OF HYPOPHYSECTOMIZED FEMALE RATS 449 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE
Photomicrographs of single glomeruli. H. and E. × 287.

FIGURE 14
UNTREATED CONTROL

FIGURE 15
GROWTH HORMONE INJECTIONS FOR 437 DAYS

A sample of skeletal muscle was taken for microscopic analysis. The animals injected with growth hormone were noted during life to be able to exert more muscular force than their controls. At autopsy it was noted that the skeletal muscle of injected rats was hypertrophied. Comparable muscle masses weighed about six or seven times as much in the experimental as in the control animal. The muscles showed histological evidence of response to growth hormone—the fibers were larger in diameter and the fibrils more substantial structures (Figs. 16 and 17).

As noted in Table 2, the heart was twice as large in experimental animals as in the control. The fibers were larger and were more distinctly separated from one another. There was a distinct increase in the vascularity. The fibrils were coarser and cross-striations were more distinct. Intercalated discs were more clearly defined. Many muscle fibers of the experimental animals contained gigantic nuclei. These nuclei were frequently four to five times as long as those of the normal cardiac muscle fiber and were frequently spiral in shape (Figs. 18 and 19).

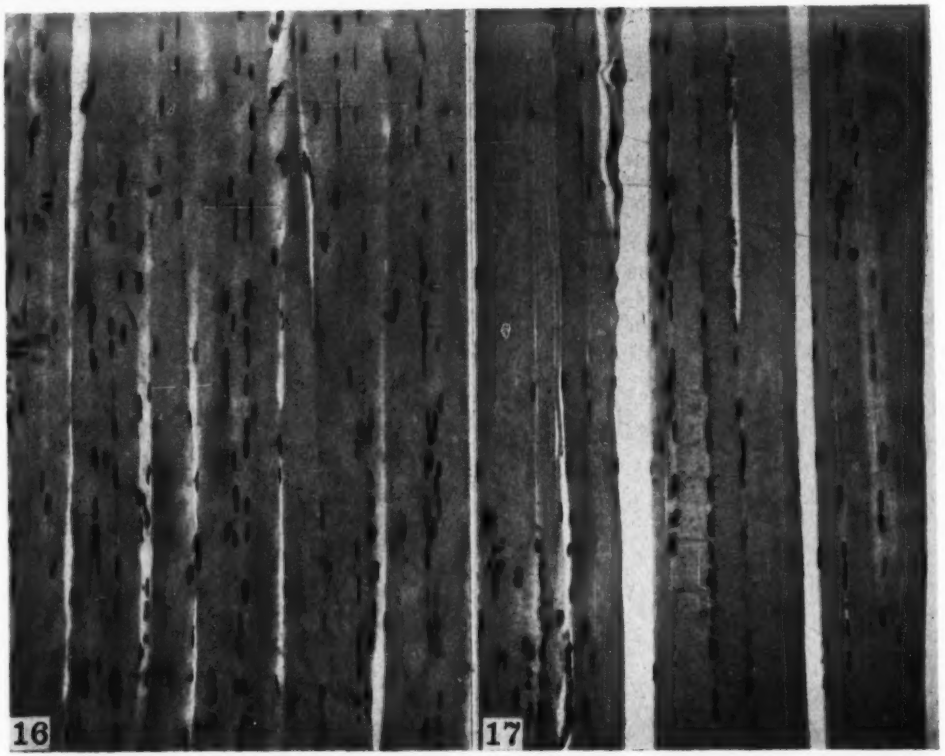
The connective tissue was tougher and it was found that the collagenous fibers were increased in size. This was perhaps most graphically portrayed in the intervertebral discs (Figs. 20 and 21).

Summary

1. Hypophysectomized rats receiving pure growth hormone from an early age grew continuously for the period of the experiment, 437 days. Both weight and skeletal dimensions increased greatly.

2. The viscera (liver, spleen, kidneys, stomach, intestine and heart) increased markedly in weight. Only the liver maintained proportionality to body weight. The brain and eyeball were scarcely increased in weight. The reproductive and endocrine organs, though slightly increased, did not show histological evidence of contamination of the growth hormone with other target organ hormones. The adrenal medulla was somewhat increased in size.

3. The individual fibers of the skeletal and cardiac musculature were hypertrophied, as was the connective tissue fiber.

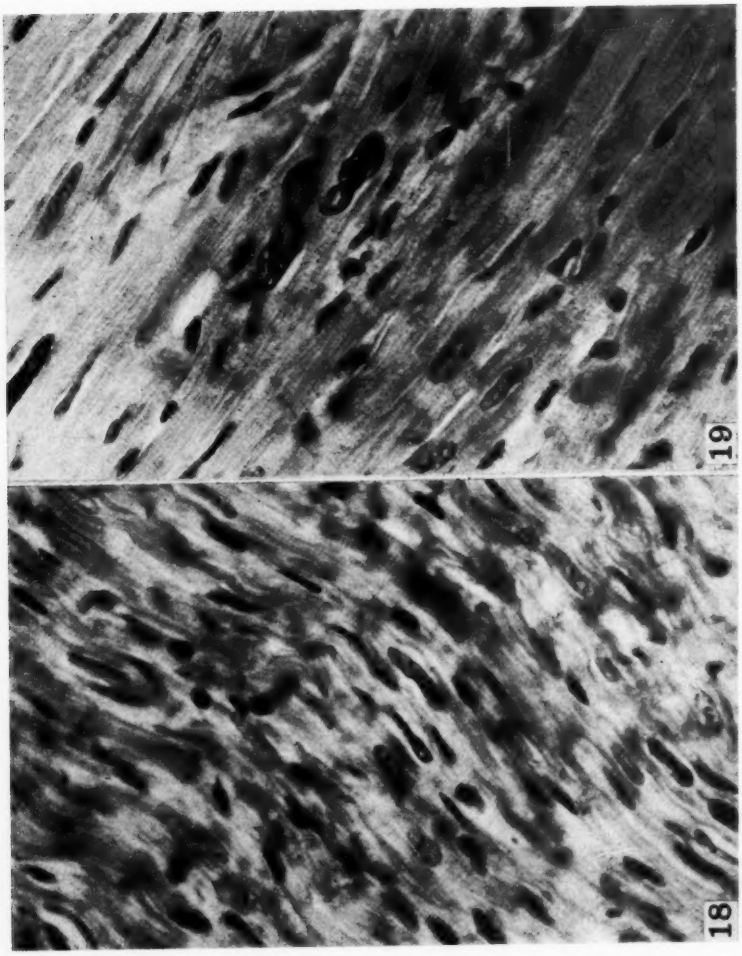


SKELETAL MUSCLE OF HYPOPHYSECTOMIZED FEMALE RATS 449 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE

Iron-hematoxylin aniline blue stain, × 196

FIGURE 16
UNTREATED CONTROL

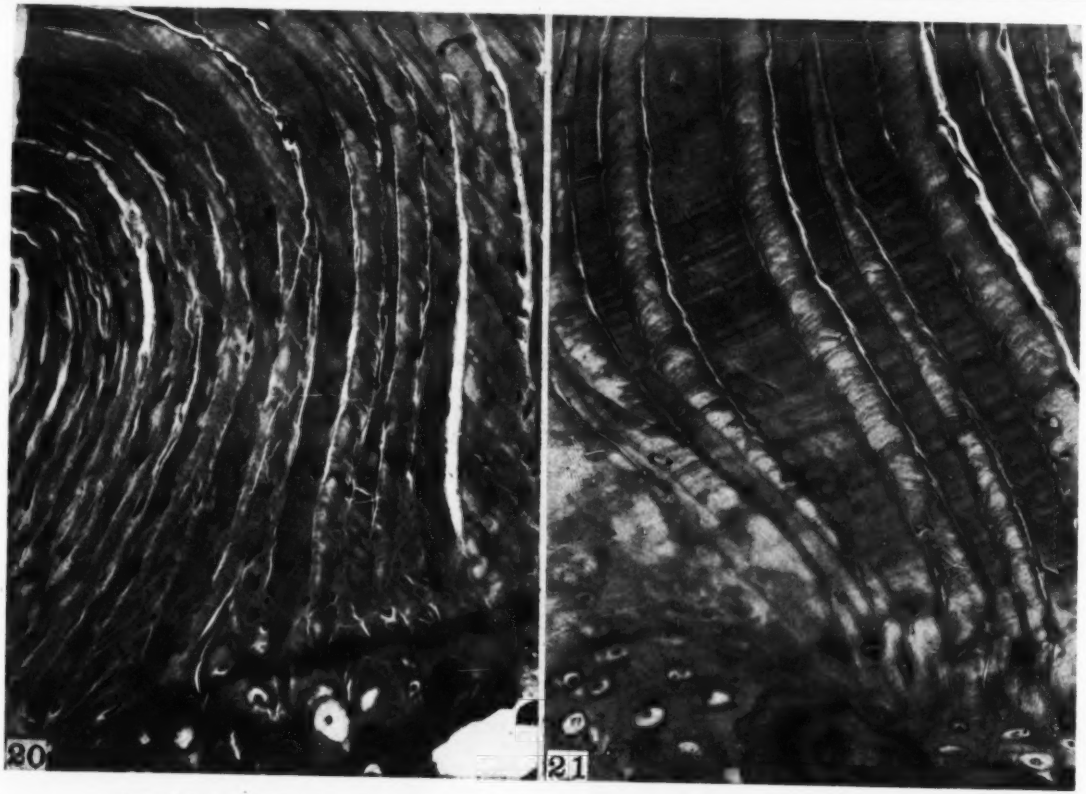
FIGURE 17
GROWTH HORMONE INJECTIONS FOR 437 DAYS



CARDIAC MUSCLE OF HYPOPHYSECTOMIZED FEMALE RATS 449 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE. H. and E. × 560.

FIGURE 18
UNTREATED CONTROL

FIGURE 19
GROWTH HORMONE INJECTIONS FOR 437 DAYS



FIBRO-CARTILAGE OF INTERVERTEBRAL DISCS FROM TAILS OF HYPOPHYSECTOMIZED FEMALE RATS, 449 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE
H. and E. × 287

FIGURE 20
UNTREATED CONTROL

FIGURE 21
GROWTH HORMONE INJECTIONS FOR 437 DAYS

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THE GROWTH OF HYPOPHYSECTOMIZED FEMALE RATS FOLLOWING CHRONIC TREATMENT WITH PURE PITUITARY GROWTH HORMONE

II. Some Chemical Components of the Musculature, Liver and Blood Plasma[1]

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The changes in the composition of the body following chronic treatment with growth hormone in normal rats have been previously reported (Li, Simpson and Evans, 1948). It was found that growth hormone induced an increase in the water and protein concentration and a decrease in the fat content. The same changes were found in the body composition of hypophysectomized animals when treated daily for thirty days (Li, Simpson and Evans, 1949). The present paper reports similar studies in hypophysectomized rats after injections of growth hormone for 437 days. In addition certain chemical components of the liver and plasma were also investigated.

An outline of the conduct of the experiment has already been given in paper I (Simpson, Evans and Li, 1949). Briefly, the plan of the experiment was as follows: 32 female rats were hypophysectomized at 26-28 days of age. Twelve days after operation the rats were divided into two groups; a group of 15 to received growth hormone, and a group of 17 being retained as controls. The treatment was continued for 437 days. The daily dosage given was as follows: 0.1 mg. for the first 111 days, 0.2 mg. for 270 days and 0.4 mg. for the last 27 days. Five animals in each group survived to the end of the experimental period.

The content of water, fat and protein in the musculature and liver was determined by procedures previously described. The blood was taken from the inferior vena cava with heparin solution as the anticoagulative agent. The method of Berenblum and Chain (1938) was

[1]Aided by grants from the American Cancer Society, Inc., New York City; The United States Public Health Service (RG-409), Washington, D. C.; The Rockefeller Foundation, New York City and the Board of Research of the University of California.

employed for phosphorus determination. The protein in the plasma was precipitated by 5.0 per cent trichloracetic acid solution; the non-protein nitrogen in the supernatant was obtained by micro-Kjeldahl analysis.

Table I presents the data on the composition of skeletal muscle. It is evident that the fat content was greatly reduced by the administration of growth hormone while the musculature of the treated animals contained more water than that of the controls. These differences are found statistically significant by the method of Fisher (1938). Although there appear no significant changes in the percentage content of protein as the result of the hormone treatment, the total amount of muscle protein in the animal is actually elevated. This is due to the increase in the muscle mass.

TABLE I

COMPOSITION OF THE MUSCLE AND LIVER AFTER CHRONIC INJECTION OF THE PITUITARY GROWTH HORMONE IN HYPOPHYSECTOMIZED FEMALE RATS

Treatment	Body Weight		Liver	Weight per 100 gm. Muscle			Weight per 100 gm. Liver	
	Initial	Final	Weight	Water	Fat	Protein	Water	Fat
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Injected[1]	68.6 ±0.5[2]	409.6 ±27.6	13.67 ±0.43	76.38 ±0.43 (<0.001)[3]	2.10 ±0.34 (<0.001)	18.94 ±0.28 (0.2)	79.62 ±0.39 (<0.001)	1.14 ±0.08 (<0.001)
Control	67.2 ±1.6	103.6 ±6.4	3.42 ±0.28	68.32 ±0.14	8.78 ±0.51	19.84 ±0.45	69.96 ±0.61	2.52 ±0.15

[1]Results based on 5 rats in each group.
[2]Figures preceded by ± are the standard errors.
[3]Fisher's p values in parentheses.

As already mentioned in paper I, the liver weight of treated hypophysectomized rats was four times that of controls. However, when the data were calculated in terms of 100 gms. body weight, the liver weight of the injected animals retained the same proportion as in the controls. Chemical analyses of these livers revealed that the fat content of the liver in the experimental animals was one-half that found in the control group. As in the case of muscle, the water content of the liver was greatly increased by the hormone injections (Table I). Thus, it is clear that the gain in body weight following the treatment with growth hormone has resulted in an accumulation

of water and protein with an accompanying loss of fat so that the composition of the tissues resembled that of chronologically younger animals.

Three chemical constituents of the plasma were carefully estimated. The non-protein nitrogen and inorganic phosphorus levels also indicated that the animals receiving continuous hormone injections were in an active growing phase. As shown in Table II, a decrease of 16 per cent of non-protein nitrogen occurred in the experimental rats, while the level of inorganic phosphorus increased from 2.5 mg. to 5.2 mg. per cent. The total nitrogen in the plasma remained unchanged.

TABLE II

TOTAL NITROGEN, NON-PROTEIN NITROGEN (NPN) AND INORGANIC PHOSPHORUS LEVELS OF PLASMA OF HYPOPHYSECTOMIZED FEMALE RATS AFTER CHRONIC INJECTION OF THE PITUITARY GROWTH HORMONE

	Body Weight		Plasma		
Treatment	Initial	Final	Nitrogen	NPN	Inorganic Phosphorus
	gm.	gm.	mg. per cc.	mg. per cc.	mg. per 100 cc.
Injected	68.6 ± 0.5	409.6 ± 27.6	9.34 ±0.46	0.357 ±0.03	5.2 ±0.41
Control	67.2 ± 1.6	103.6 ± 6.4	9.20 ±0.45	0.424 ±0.02	2.7 ±0.20

SUMMARY

Estimates were made of the chief chemical components of the muscle, liver and plasma of hypophysectomized rats after treatment with the anterior hypophyseal growth hormone for a period of 437 days. The results indicate that the changes in the protein, water and fat content of the muscle and liver and in the inorganic phosphorus and non-protein nitrogen levels of the plasma are those characterizing youthful tissues and are consonant with the concept that the hormone has occasioned true growth.

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THE GROWTH OF HYPOPHYSECTOMIZED FEMALE RATS FOLLOWING CHRONIC TREATMENT WITH PURE PITUITARY GROWTH HORMONE[1]

III. Skeletal Changes: Tibia, Metacarpal, Costochondral Junction and Caudal Vertebrae

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In this study, the third in a series of reports on the growth of hypophysectomized rats injected with pure anterior pituitary growth hormone for a prolonged period, the objective is to describe the skeletal changes. A previous paper reported the skeletal characteristics in the gigantism developed by normal rats injected with growth hormone for a similar period (Evans, Becks, Asling, Simpson and Li, 1948). This paper parallels the previous study in that it presents measurements and a description of the histology of the tibia, costochondral junction and caudal vertebrae. In addition, the third metacarpal is described. This bone, unlike most of the long bones of the rat, has an early age of epiphyseal closure (100 ± 10 days), and hypophysectomy results in the failure of this closure to occur. The examination of the metacarpal was therefore advantageous since it might be determined whether the growth hormone would maintain the epiphyseal plate in an intact and active state, and thus prolong the period of growth of the bone. The description of the mandible and the dentition is reserved for a separate report.

The experimental conditions have been described in the first paper of the present series (Simpson, Evans and Li, 1949). In recapitulation, they were as follows: female rats of the Long-Evans strain were hypophysectomized at 26 to 28 days of age. Ten of these survived

[1]Aided by grants from the American Cancer Society, Inc., New York City; The U. S. Public Health Service (RG-409 and RG-800R); The American Foundation for Dental Science, The California State Dental Association and the Research Board of the University of California, Berkeley.

the full experimental period and were sacrificed 449-462 days after operation, their ages then being 475 to 488 days. Five of the rats were controls; the other five had received injections of pure pituitary growth hormone for 437 days, starting 12 days after hypophysectomy. The preparation of growth hormone had a potency of 50 units per milligram, determined by the 10-day weight gain test in hypophysectomized rats. The hormone was injected 6 times weekly. The initial daily dose was 0.1 mgm.; this was increased with increasing body weight until a final daily dose of 0.4 mgm. was being given.

At autopsy the carcasses were pinned out flat in an extended position on paraffin slabs and fixed in 10% neutral formol. They were roentgenographed, and the selected bones then prepared for histologic study.

Results

The measurements of the tibia, metacarpal, and caudal vertebrae are given in Table I. The figures show that in all dimensions measured the injected rats significantly exceeded their hypophysectomized controls. The individual figures will be considered in connection with the roentgenographic and histologic studies of the bones to which they pertain.

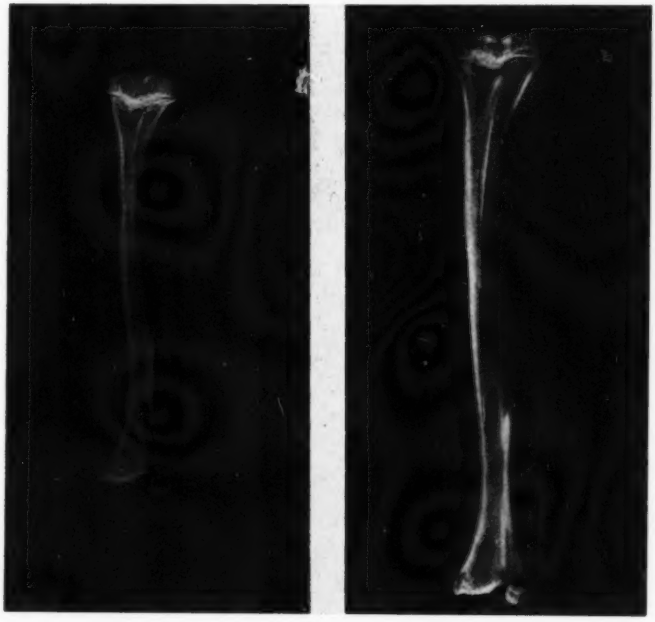
TABLE I

Dimensions of the Tibia, Metacarpal, and Caudal Vertebrae of Rats Hypophysectomized and Injected with Growth Hormone for 437 Days and of Their Uninjected Hypophysectomized Controls*

Structures Measured	Dimensions in Millimeters	
	Uninjected Group	Growth Hormone Injected Group
Tibia, length	30.8 ± 0.63	43.4 ± 0.64
dorsoventral diameter of shaft	4.8 ± 0.05	6.5 ± 0.17
mediolateral diameter of shaft	2.0 ± 0.04	2.8 ± 0.16
Metacarpal, length	6.6 ± 0.07	7.7 ± 0.11
Tail, length (anus to tip of tail)	127 ± 2.4	182 ± 3.8
9th Caudal Vertebra, length	6.6 ± 0.14	9.3 ± 0.19
19th Caudal Vertebra, length	4.0 ± 0.11	6.1 ± 0.14

*Averages and standard errors of 5 rats per group.

Tibia. Figures 1 and 2 show roentgenograms of the tibias of an uninjected and an injected hypophysectomized rat. The increased dimensions of the bone of the rat receiving growth hormone are easily seen. The average length of the tibias of uninjected rats was 30.8 mm.; that of the injected rats was 43.4 mm.[2] (Table I). The



ROENTGENOGRAMS OF DISSECTED TIBIAS AFTER FORMOL FIXATION. × 2

FIGURE 1
Uninjected hypophysectomized rat 449 days after operation at 26 days of age. Sp. 9019, Pl. B181.

FIGURE 2
Growth hormone treated hypophysectomized rat, injected for 437 days, beginning 12 days after the operation at 28 days of age. Sp. 9025, Pl. B182.

[2]The average length of the tibias of the injected rats surpassed that of normal adults. There was no overlap in the range. The shortest of this group was 41.8 mm. whereas the longest tibia found in normal female rats of this strain was 41.6 mm. The tibias of uninjected hypophysectomized rats must have grown somewhat after the operation, since at autopsy their length was greater than that normal for 28 days of age. However, their length only reached that normal for 44 days of age. (Average tibia lengths of normal rats at representative ages are as follows: 28 days of age, 25.2 ± 0.17 mm.; 44 days, 30.4 ± 0.33 mm., 250 days, 39.6 ± 0.51 mm. It is the same at 650 days of age. That of a 1316 day old rat (40.2 mm.) was within this range.

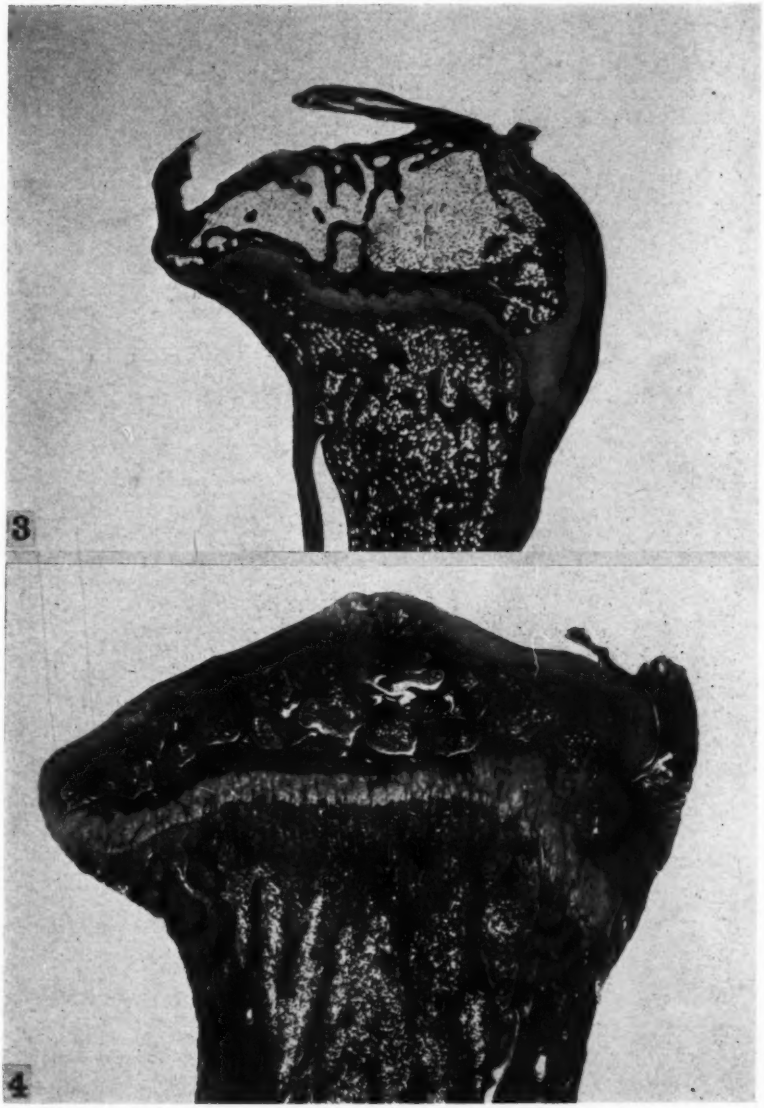
diameters of the head and the shaft were also significantly greater in the injected rats than in controls, as is seen from the roentgenograms and the table.

The two groups of rats showed marked differences in the histological structure of their tibias. In the uninjected hypophysectomized rats there was found the atrophy characteristic for the proximal end of the tibia at a long interval after hypophysectomy. The main features were small size, thin cortical bone, narrow epiphyseal cartilage plate (average width $189 \pm 7.3\ \mu$), and adipose marrow. The injected hypophysectomized rats' tibias showed numerous evidences of prolonged growth activity. The bones were large, the cortical bone was thicker and perforated by many vessels, and the epiphyseal cartilage plate was wider (average width $293 \pm 9.4\ \mu$). The marrow was cellular.

Figures 3 and 4 are photomicrographs at low magnification of sections through the proximal end of the tibia in rats of the two groups. In the uninjected hypophysectomized rat (Fig. 3) a thin layer of tissue undistinguishable from fibrous tissue is seen on the articular surface. The cortical bone of the epiphysis is thin and forms a continuous layer surrounding the marrow cavity. Only a few bony trabeculae are present. The marrow cavity is predominantly adipose. On the diaphyseal side of the narrow cartilage plate the bony trabeculae are sparse, short, coarse, and anastomosing. The cortical bone of the diaphysis is thin and shows few vessels; it is arranged in lamellae parallel to the surface.

In the injected hypophysectomized rat (Fig. 4)) a thick layer of cartilage covers the articular surface of the head of the tibia. Its chondrocytes are numerous and when examined under higher magnification showed a columnar arrangement. Islands of cellular marrow interrupt the bony layer adjacent to this cartilage. In the diaphysis, subjacent to the epiphyseal cartilage plate, the bony trabeculae are numerous, delicate, and straight. The marrow is predominately cellular. The cortical bone is thick and highly vascular. The outer (sub-periosteal) layer lies in lamellae parallel to the periosteum, while the inner layer is thick, vascular, and organized into Haversian systems.

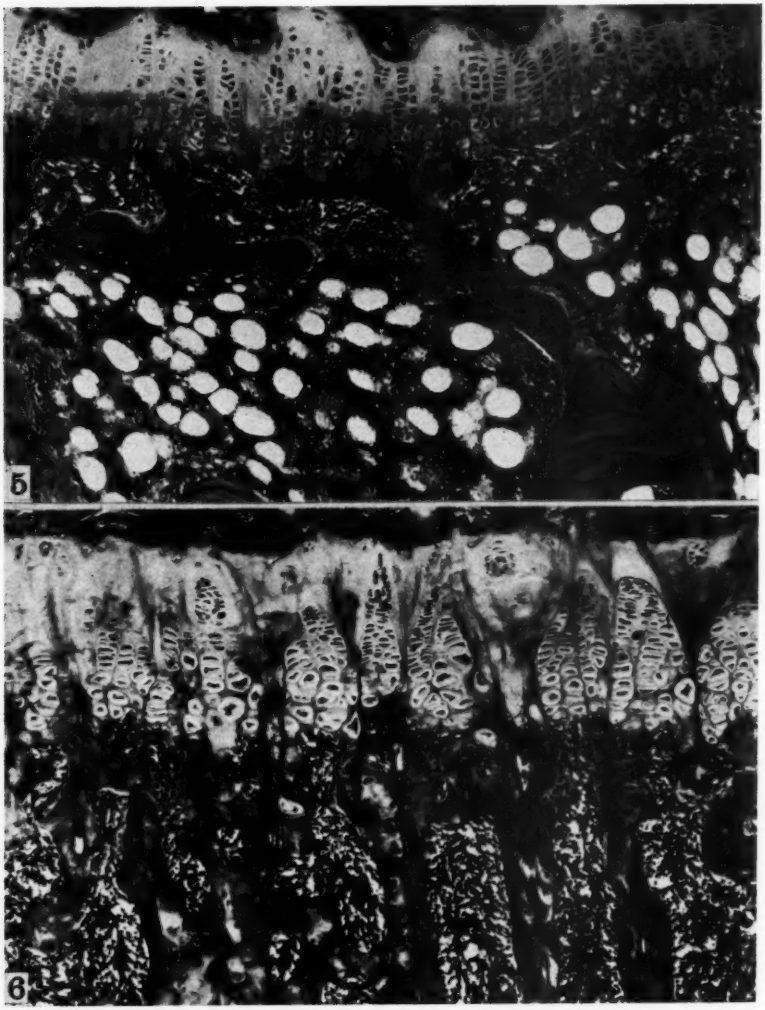
Examination of the region of the epiphyseal cartilage plate under higher magnification made it even clearer that the balance between cartilage formation and osseous replacement in the injected rats resembled that seen in the normal growing rat (Becks, Simpson and Evans, 1945a). The cartilage plates showed active chondrogenesis, continuing erosion of the cartilage at its diaphyseal surface, and the formation of multiple diaphyseal bony trabeculae. In contrast, these proximal epiphyseal cartilage plates of uninjected rats showed almost complete cessation of chondrogenesis and osteogenesis, and corresponded closely to the condition previously described as characteristic in this bone at long post-operative intervals (Becks, Simpson and Evans, 1945b).



PHOTOMICROGRAPHS OF MEDIAN SECTIONS OF THE PROXIMAL EPIPHYSEAL REGION OF THE TIBIA. H. and E. × 15

FIGURE 3
Uninjected hypophysectomized rat 462 days after operation at 26 days of age. Sp. 9021, Pl. 9885.

FIGURE 4
Growth hormone treated hypophysectomized rat, injected for 437 days, beginning 12 days after the operation at 26 days of age. Sp. 9024, Pl. 9884.



PHOTOMICROGRAPHS AT HIGHER MAGNIFICATION OF MEDIAN SECTIONS OF THE REGION OF THE PROXIMAL EPIPHYSEAL PLATE OF THE TIBIA FROM THE ANIMALS SHOWN IN FIGURES 3 AND 4. H. and E. × 113

FIGURE 5
Uninjected hypophysectomized rat. Pl. 9887.

FIGURE 6
Growth hormone treated hypophysectomized rat. Pl. 9886.

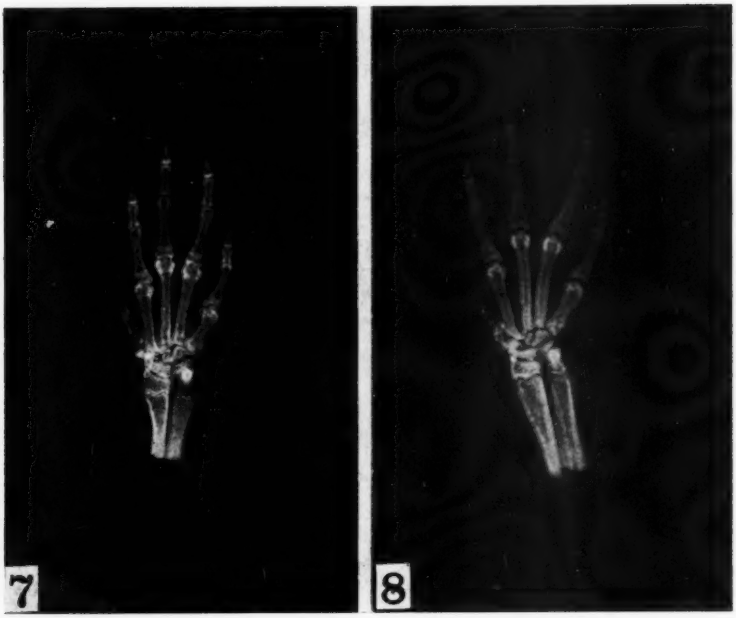
Figures 5 and 6 show, at high magnification, the epiphyseal cartilage plates of an uninjected and an injected hypophysectomized rat. In Fig. 5 small chondrocytes are seen throughout the cartilage, often in rows extending through its full width. Near the marrow junction the cells are rounded, and the matrix shows the basophilia of calcification. Transverse segments of bone lie against the surface of this cartilage and seal it almost completely from the marrow of the diaphysis. Though marrow occasionally lies in contact with the cartilage, there is no evidence of erosion. Sparse, short bony trabeculae are seen in this marrow cavity.

In Figure 6 the features characteristic of the epiphyseal plate in the injected rats are shown. While chondrogenesis is active, the clusters of large cells are mainly confined to the half of the plate toward the diaphysis. Wedges of matrix containing convergent basophilic striae separate these coniform clusters. Marrow tufts impinge upon the groups of cartilage cells. Between these groups the matrix of the wedges extends directly into trabeculae. Osteoblasts are distinguishable on the surfaces of these trabeculae; they were enlarged to varying degrees in the different animals of the group and were observed with special frequency in the buttress areas and on the endosteum of the cortical bone.

It has thus been demonstrated that at the proximal end of the tibia, where epiphyseal closure is normally delayed until senescence, the epiphysis in injected rats retained the capacity for growth throughout the experimental period. The growth of the tibia is, however, partially dependent on another epiphysis, the distal, which normally closes early (Dawson, 1925). In this experiment it closed during the injections of growth hormone, although in the uninjected hypophysectomized rats a sealed-off, atrophic cartilage plate persisted throughout the experimental period. In this respect the behavior of this epiphysis was so like that of the metacarpal (next to be described in detail) that further description of the distal end of the tibia has been omitted.

Metacarpal. The roentgenograms reproduced in Figure 7 and 8 illustrate the forepaws of rats from the two groups. The average lengths of the third metacarpal are seen in Table I. In the injected rats this length, 7.7 mm., significantly exceeded that in the uninjected rats, 6.6 mm.[3] The metacarpal is characterized by the development of only one epiphyseal cartilage plate, at its distal end. The status of this epiphyseal cartilage plate could be determined from the roentgenograms. In the uninjected hypophysectomized rats this plate was still present at the end of the experiment. This persistence, long after the normal time of disappearance, is characteristic for the hypophysectomized rat (Collins, Becks, Asling, Simpson and Evans, '48). In the

[3]The metacarpals of the injected rats attained but did not exceed normal length. In the uninjected rats this bone was longer than at the time of hypophysectomy, but it had not attained even the length normal for 40 day old rats. The normal length at 26 days of age is 6.3 ± 0.04 mm., at 40 days is 7.0 ± 0.11 mm., and at 70 days, 7.6 ± 0.09 mm. (the definitive length).



ROENTGENOGRAMS OF FOREPAWS AFTER FORMOL FIXATION. ×2

FIGURE 7

Uninjected hypophysectomized rat 461 days after operation at 26 days of age. Sp. 9020, Pl. B183.

FIGURE 8

Growth hormone treated hypophysectomized rat, injected for 437 days beginning 12 days after the operation at 28 days of age. Sp. 9025, Pl. B184.

injected rats this cartilage plate disappeared during the injection period.[4]

Histological studies made at the termination of the experiment confirmed the finding that cartilage plates no longer existed in the injected rats. In two of the five rats fragments of cartilage were present. Such masses of cartilage seldom persist in the normal meta-

[4]After 246 days of injection, just past the mid-point of the 437 day injection period, the two largest injected rats were anesthetized and roentgenographed. On comparison of these roentgenograms with the terminal records of the same rats, the following lengths and status of epiphyseal cartilage plate were found.

Animal	Length of third metacarpal		Epiphyseal plate	
Number	Interval	Terminal	Interval	Terminal
B9846	7.4 mm.	7.6 mm.	Present	Absent
GH9927	7.8 mm.	7.9 mm.	Absent	Absent

carpal after epiphyseal closure. The cartilage plates in the hypophysectomized controls showed no histological evidence of chondrogenesis and osteogenesis. The cartilage plate was sealed from the marrow cavities of both epiphysis and diaphysis by bone.

Costochondral Junction. A marked lateral displacement of the costochondral junction was found in roentgenograms of the thoracic cages of the injected rats. An explanation of this disproportion was sought in histologic studies of this junction. In the uninjected hypophysectomized rats virtually no evidences of activity, either chondrogenic or osteogenic, were found, and the rib was thin and delicate. In the injected rats there were evidences of cartilage proliferation, but active osteogenesis was not seen. This failure of the bony growth of the rib to keep pace with cartilaginous growth probably accounted for the displacement of the costochondral junction.

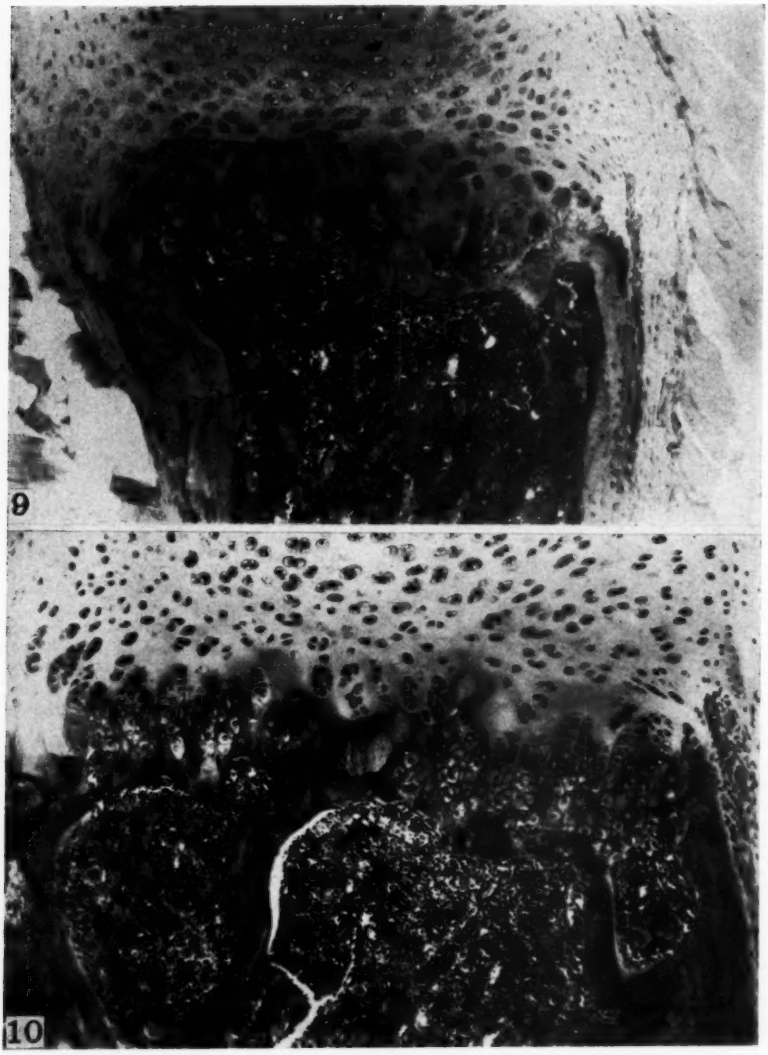
Figures 9 and 10 illustrate the costochondral junction in rats of the two groups. In the uninjected hypophysectomized rat (Figure 9) the chondrocytes are small and lie in clusters in an abundant matrix. Those near the marrow cavity are somewhat rounded but show little enlargement or vacuolation. The line marking off the cartilage from the medullary cavity of the rib is regular and is marked by a narrow layer of sealing-off bone. The marrow cavity is devoid of bony trabeculae and contains a cellular marrow.

In the injected rat (Figure 10) there are evidences that chondrogenesis has been active near the costochondral junction. The whole region is broader, the cartilage is more cellular, and the matrix is scanty. While these features do not indicate an activity as great as that characterizing rapid growth, it is apparent that an appreciable amount of cartilage proliferation has been maintained in the injected rats. There is, however, no evidence of active osteogenesis. The junction of the cartilage with the marrow is irregular, and bears a layer of bone. Although this bone is interrupted at some points, and marrow thus lies in contact with the cartilage, no capillary tufts are seen. Few bony trabeculae are present.

Caudal Vertebrae. Roentgenograms of the tails of hypophysectomized control and injected rats are reproduced in Figures 11 and 12 respectively. Table I records the average lengths of the entire tail, and of the 9th and the 19th caudal vertebrae. The average length of the tails of uninjected rats was 127 mm. and of injected rats, 182 mm. The 9th caudal vertebra (uninjected 6.6 mm., injected rats 9.3 mm.) and the 19th caudal vertebra (uninjected 4.0 mm., injected rats 6.1 mm.) showed the same degree of difference.[5]

The number of caudal vertebrae present was the same in control and injected groups, 27 to 30 segments. However, a difference was

[5]The 9th caudal vertebra of these injected rats had attained normal dimensions, while the 19th was slightly shorter than normal. In 650 day old normal rats these vertebrae are 9.3 ± 0.21 mm. and 6.6 ± 0.19 mm. respectively.



PHOTOMICROGRAPHS OF SECTIONS OF THE COSTOCHONDRAL JUNCTION FROM THIRD LEFT RIBS. H. and E. × 124

FIGURE 9

Uninjected hypophysectomized rat 449 days after the operation at 26 days of age. Sp. 9019, Pl. B171

FIGURE 10

Growth hormone treated hypophysectomized rat, injected for 437 days, beginning 12 days after the operation at 26 days of age. Sp. 9023, Pl. 9894.

ROENTGENOGRAMS OF TAILS, AFTER FORMOL FIXATION AND REMOVAL OF SKIN.
¾ Natural size.

FIGURE 11
Uninjected hypophysectomized rat 461 days after the operation at 26 days of age. Sp. 9020, Pl. B185.

FIGURE 12
Growth hormone treated hypophysectomized rat, injected for 437 days, beginning 12 days after the operation at 26 days of age. Sp. 9023, Pl. B186.

found in the differentiation of the distal segments. In the majority of the injected rats all of the caudal vertebrae showed two epiphyseal ossification centers roentgenographically. All hypophysectomized controls showed less differentiation of the most distal segments; in one rat the last four segments lacked an epiphyseal calcification.

Histologically the outstanding features of the caudal vertebrae as seen at low magnification were the greater dimensions and the increased amount of trabecular and cortical bone seen in the injected rats (Figures 13 and 14). The cortical bone, especially, was thick and vascular. Lines of bony accretion were found along the blood vessels of this bone, as well as sub-periosteally and sub-endosteally. When the region of the epiphyseal plate of the vertebrae was examined at higher magnification the injected rats showed evidences of chondrogenesis and osteogenesis which, while not as active as in young growing rats, were distinctly more active than in the controls. There appeared to be a normal balance between cartilage proliferation, erosion, and bone formation. The marrow was adipose as in the controls.

Figures 15 and 16 illustrate the region of the proximal epiphyseal cartilage plate of the 9th caudal vertebra from rats of each group. In the photomicrograph from the uninjected rat (Figure 15), the cartilage is narrow and irregular in width; toward the marrow cavity of both epiphysis and diaphysis it is basophilic. The cartilage is not completely sealed from the diaphysis by bone, but short "caps" of bone are seen on its surface.

As seen in Figure 16 the cartilage plate from the injected rat is uniformly wider. Its cells are slightly larger and more numerous. The matrix is more uniform and is still basophilic; no vacuolated cells are seen. Marrow is in contact with some cartilage cells, and bony trabeculae extend into the diaphysis. Many of these trabeculae show large cores of atrophic cartilage with a thin bony layer on the surface. Lines of bony accretion are distinguishable on the surfaces of some of the trabeculae.

Summary

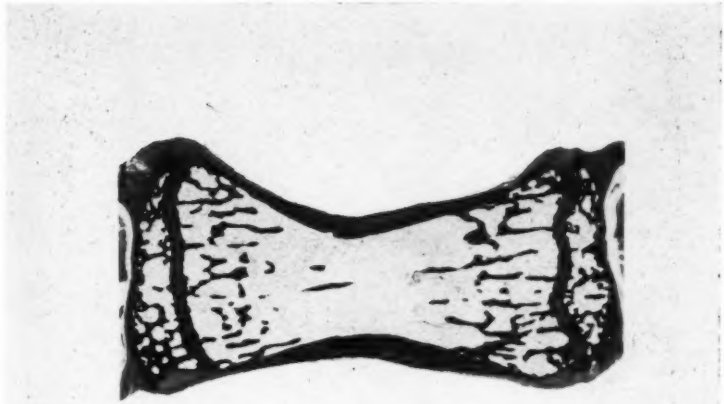
The injection of pure growth hormone for 14 months in hypophysectomized rats resulted in marked growth of all skeletal dimensions studied. The length of the tibia actually exceeded normal, while the metacarpals and the caudal vertebrae equalled normal length. The bones also grew in breadth.

The proximal end of the tibia showed histological evidences of continued growth, with a balance between chondrogenesis and osteogenesis resembling that found in normal rats at younger ages.

The cartilage plate of the distal end of the tibia and of the metacarpal disappeared during the injections. In the latter bone its contribution to the attainment of normal dimensions was demonstrated.

In the rib little osteogenesis was seen at the costochondral junction, but chondrogenesis must have continued longer than osteogenesis, judged by the lateral displacement of this junction seen in roentgenograms.

In the caudal vetebrae the activity at epiphyseal cartilage plates,



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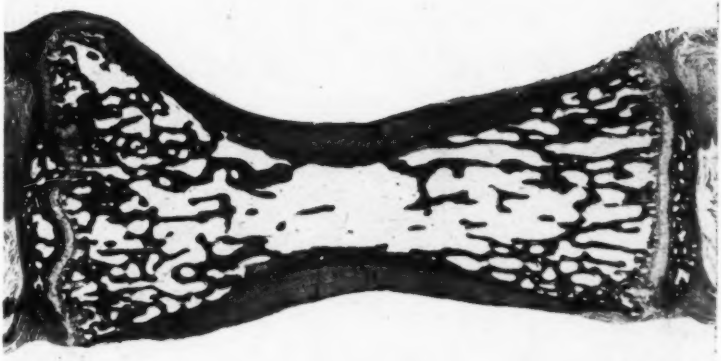

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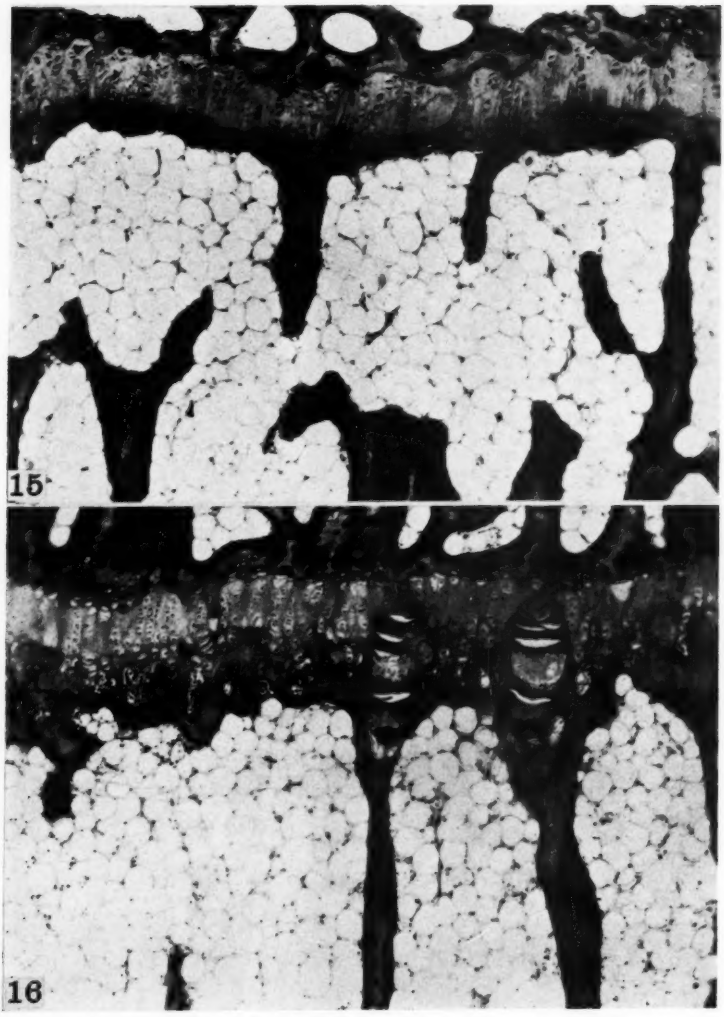
PHOTOMICROGRAPHS OF MEDIAN SECTIONS OF THE NINTH CAUDAL VERTEBRA FROM THE ANIMALS SHOWN IN FIGURES 12 AND 13. H. and E. × 16

FIGURE 13

Uninjected hypophysectomized rat. Pl. 9724.

FIGURE 14

Growth hormone treated hypophysectomized rat. Pl. 9722.



PHOTOMICROGRAPHS OF THE PROXIMAL EPIPHYSEAL REGION FROM NINTH CAUDAL VERTEBRAE. H. and E. × 113

FIGURE 15

Uninjected hypophysectomized rat 461 days after the operation at 26 days of age. Sp. 9020, Pl. 9888.

FIGURE 16

Growth hormone treated hypophysectomized rat, injected for 437 days, beginning 12 days after the operation at 26 days of age. Sp. 9024, Pl. B172.

while slow, indicated a balance between chondrogenesis and osteogenesis. Additional epiphyseal ossification centers were developed in the terminal segments during the injection of growth hormone.

All bones examined histologically showed lines of accretion under the periosteum indicating that periosteal osteogenesis was stimulated.

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THE GROWTH OF HYPOPHYSECTOMIZED FEMALE RATS FOLLOWING CHRONIC TREATMENT WITH PURE PITUITARY GROWTH HORMONE[1]

IV. Skeletal Changes: Differences in Response from That of Intact Rats

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The histologic examination of selected sites of endochondral ossification (tibia, costochondral junction, metacarpal, and caudal vertebrae) in hypophysectomized rats chronically injected with growth hormone, demonstrated differences in the reactivity of these various centers which led to disproportionate growth (Becks, Asling, Simpson, Li and Evans, 1949). Inspection of roentgenograms disclosed such disproportions throughout the body. It was noteworthy that, while the skeletons of these injected rats attained normal dimensions, certain parts of the skeleton equalled or even exceeded the dimensions of the corresponding bones in intact rats injected for the same period (Evans, Becks, Asling, Simpson and Li, 1948). Disproportionate growth resulting from growth hormone seemed more frequent in the hypophysectomized rats than in the intact rats. Analyses of roentgenograms of these animals have therefore been made, to determine the extent of these shifts in body proportions resulting from the chronic injection of growth hormone in normal and in hypophysectomized rats.

Figures 1 to 4 are reproductions of roentgenograms of typical rats from these 4 groups. Figure 1 shows a female rat 449 days after hypophysectomy at 26 days of age. Figure 2 shows a hypophysectomized rat of similar age, injected with growth hormone for 437 days.

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Figure 3 shows a normal female rat 650 days of age. Figure 4 shows a normal female rat of similar age, injected with growth hormone for 437 days. Details of the care and treatment of these animals have been described elsewhere (Evans, Simpson and Li, 1948; Simpson, Evans and Li, 1949).

From these roentgenograms there may be seen many of the differences in body dimensions and proportions which characterized the rats of the various groups. The following features are of special interest.

Axial skeleton. In the uninjected hypophysectomized rats the skull though small was relatively broader and shorter than in the normal rats, having retained many of the proportions of immaturity. The injection of growth hormone accentuated this difference from the normal adult. The injected hypophysectomized rat was remarkable for the breadth and massiveness of the zygomatic arch. The great development of this arch may be associated with the muscular hypertrophy which caused the heads of these rats to appear so thick during life. The ruggedness of the tympanic bullae and paramastoid processes which caused the caudal ends of these skulls to appear squared rather than tapering may also have been associated with muscular development. The atlas, too, was massive. The false ribs of these rats were flared and everted. Though not seen in roentgenograms, the marked eversion of the xyphoid process of rats of this group received special comment at autopsy. The ribs showed narrow necks and abrupt broadening to shafts of great width and complex trabecular structure. The costal cartilages were broad and mottled.[2] The costochondral junctions were displaced so far laterally as to be obscured by the shadow of the lateral borders of the thoracic cage, rather than appearing in the more ventral position characteristic of the other three groups. The transverse processes of the lumbar vertebrae were directed cephalad so that they lay parallel to the bodies of the vertebrae, rather than inclining from these vertebrae at a distinct angle as in the rats of the other three groups.

Appendicular skeleton. Roentgenographically, marked differences were seen in the girdle bones as well as the long bones of the rats in the four groups. The scapulae of the injected hypophysectomized rats were longer and narrower and the pelvis was tubular. The long

[2]In histologic sections, the central portions of these costal cartilages were intensely basophilic, and their characteristic architecture was disrupted.

ROENTGENOGRAMS OF DISSECTED SKELETON

FIGURE 1
Hypophysectomized female rat 449 days after operation at 26 days of age. Sp. 9017.

FIGURE 2
Hypophysectomized female rat of similar age injected with growth hormone for 437 days, beginning 2 weeks after the operation. Sp. 9023.

ROENTGENOGRAMS OF ANIMALS UNDER ANESTHESIA

FIGURE 3
Normal female rat 650 days of age. Sp. 8979.

FIGURE 4
Normal female rat of similar age, injected with growth hormone for 437 days. Sp. 8984.

bones of these rats surpassed in size and density not only those of their uninjected hypophysectomized controls but often exceeded those of uninjected normal rats. The deltoid tuberosity of the humerus of the injected hypophysectomized rats attained a prominence not seen in the other groups. The fore-paws of injected rats, both hypophysectomized and intact, did not grow beyond normal size.

An analysis of the persistence or absence of cartilage plates as determined in roentgenograms at representative sites of endochondral ossification[3] is given in Table I. These bones have been listed in a

TABLE I

PERSISTENCE OR ABSENCE OF THE EPIPHYSEAL CARTILAGE PLATES IN HYPOPHYSECTOMIZED AND NORMAL RATS, UNINJECTED AND INJECTED CHRONICALLY WITH GROWTH HORMONE*

Site of Cartilage Plate**	Normal age of Closure (days)	Hypophysectomized (age 480 days)		Normal (age 650 days)	
		Uninjected	Injected	Uninjected	Injected
Humerus (distal)[8]	31-42	—	—	—	—
Radius (proximal)[10]	83-92	+	+—	—	—
Calcaneus	84-119	+	—	—	—
Ilium, junction with ischium and pubis	84-119	+	—	—	—
Tibia (distal)[10]	92-98	+	—	—	—
Metacarpal III[4]	100	+	—	—	—
Ulna (olecranon)[10]	940	+	+	+	+
Tibia (proximal)[10]	1135	+	+	+	+
Humerus (proximal)[10]	1135+	+	+	+	+
Radius (distal)[10]	1135+	+	+	+	+
Ulna (distal)[10]	1135+	+	+	+	+
Fibula (proximal)[10]	1135+	+	+	+	+

*The symbols used to represent the status of the epiphyseal cartilage plate are as follows: + indicates presence of epiphyseal cartilage plate; — indicates that these centers have fused; + — indicates variability within the group.

**Data on age of closure from references in literature cited indicated by superscript; remainder unpublished.

[3]It has been shown previously (Becks, Asling, Collins, Simpson, and Evans, '48) that a high degree of accuracy may be attained in roentgenographic studies of bones, both as regards the status of epiphyses and the lengths of the bones. A fine-grain film is necessary for such studies; the film used (Eastman Industrial Type A X-ray Film) has a finer grain than the films usually employed in clinical roentgenography. A 36-inch tube distance was employed in making the roentgenograms, in order to minimize distortion of the length of the roentgenographic shadows through parallax. Precision is increased by using a dissecting microscope in inspection of the roentgenograms. Under such conditions roentgenographic determinations of the status of epiphyseal lines agreed closely with those established from histologic sections. Measurements of the length of the metacarpal bone were the same when determined in histologic sections or in roentgenograms. Measurements of length of the freshly dissected tibia correspond to those made on roentgenograms of the same bones. In some cases, particularly the clavicle, humerus, and femur, the figures in Table 2 may represent less than the actual measurement, due to the foreshortening of roentgenographic shadows resulting from the angulation of these bones. This, however, being a constant factor in the positioning of the animals for roentgenography, the relative values may still be compared.

sequence corresponding to their normal time of epiphyseal closure. It will be seen that in rats hypophysectomized at 26-28 days of age epiphyseal fusion occurred during prolonged injection of growth hormones at those epiphyses which normally close early (80-120 days), whereas these plates persisted in the uninjected hypophysectomized rats. Such, for example, was the case at the epiphysis of the third metacarpal, the distal epiphysis of the tibia, the calcaneus, and the junction between the ilium and the ischium-pubis (triradiate cartilage). Those epiphyses which normally remain separate until the senescent period did not fuse in the injected animals. In short, in the injected hypophysectomized rats the status of the epiphyses studied was brought to that characterizing normal rats. The status of the epiphyses of normal rats was not changed by the injection of growth hormone.[4] In neither group did premature epiphyseal closure attend the chronic injection.

Representative parts of the skeleton were measured in roentgenograms[3] in order to make quantitative the comparisons of the distinctive features of the various groups. Additional measurements were made to detect differences in proportion not obvious on inspection. Table II shows the measurements made and their average values and standard errors in the different groups.

The hypophysectomized controls differed from normal adult rats in that they were dwarfed in all dimensions. This dwarfism was disproportional, the individual bones varying in the percent of normal adult size which they attained. For example, the pelvic width (at the acetabulum) was 62% of normal, the total body length 70%, the tibia length 78%, and the metacarpal length 88% of normal.

The injected hypophysectomized rats were compared with normal rats to determine whether the disproportions of the hypophysectomized control were corrected or further exaggerated. The dimensions

[4]It should be noted that the injection period of the normal rats receiving growth hormone did not start until these animals were 180-210 days of age. It is therefore obvious that those epiphyses known to fuse before this age could not have had their closure affected by the experimental treatment. However, it is unlikely that starting the injection period earlier would have influenced closure at such epiphyses, since growth hormone has been shown to be without effect on the time of epiphyseal closure of the metacarpal bone (Asling, Becks, Simpson, Li, and Evans, '48).

In hypophysectomized rats, epiphyseal ossification centers and their associated cartilage plates were present at all of the sites studied at the time injections began, with the exception of the distal epiphysis of the humerus (Sparks and Dawson, '28; Strong, '26). At this site closure takes place early (35 days) and is not affected by hypophysectomy at 26-28 days (Becks, Asling, Simpson, Evans, and Li, '48).

TABLE II

MEASUREMENTS OF THE SKELETON OF HYPOPHYSECTOMIZED AND NORMAL RATS, UNINJECTED AND INJECTED CHRONICALLY WITH GROWTH HORMONE

	Hypophysectomized (age 480 days)		Normal (age 650 days)	
	Uninjected	Injected	Uninjected	Injected
	mm.	mm.	mm.	mm.
AXIAL SKELETON				
Body Length				
Total	279±3.3	392±7.2	405±9.3	454±6.0
Nose-Anus	152±1.5	210±3.8	219±4.2	243±2.8
Anus-Tail Tip	127±2.4	182±3.8	187±5.2	211±2.8
Skull				
Length	36.7±0.41	43.4±1.63	43.4±0.47	49.3±0.64
Width	19.2±0.71	27.0±0.54	23.1±0.39	27.4±0.32
Cranium				
Length	19.2±0.33	22.5±0.38	23.9±0.83	26.1±0.48
Width	15.6±0.32	20.0±0.54	17.1±0.38	19.3±0.25
Thorax				
Length	37.6±1.31	56.7±1.23	56.2±1.32	67.0±1.67
Width (level of 1st rib)	9.5±0.11	13.9±0.23	12.7±0.33	14.5±0.18
Width (level of 10th-11th rib)	38.7±1.18	57.8±1.30	50.8±1.64	61.3±2.90
Vertebrae				
Length, 5th lumbar	4.6±0.10	7.0±0.15	6.9±0.15	8.3±0.37
Length, 9th caudal	6.6±0.14	9.3±0.19	9.3±0.21	10.6±0.25
Length, 19th caudal	4.0±0.11	6.1±0.14	6.6±0.19	7.5±0.20
Width, 1st lumbar	4.9±0.04	7.1±0.11	6.2±0.09	6.7±0.10
APPENDICULAR SKELETON				
Pectoral girdle				
Scapula, length	17.2±0.65	29.9±0.64	23.6±0.86	26.9±0.61
Scapula, width	6.9±0.21	11.3±0.37	9.9±0.42	12.9±0.39
Clavicle, length	9.8±0.34	13.2±0.13	14.1±0.23	16.4±0.57
Fore-limb				
Humerus, length	19.2±0.53	27.9±0.63	27.8±0.50	32.0±0.37
Humerus, width of deltoid tuberosity	1.8±0.10	3.7±0.12	2.1±0.05	2.9±0.11
Radius, length	18.9±0.35	26.9±0.35	25.8±0.34	29.1±0.49
Ulna, length	24.3±0.29	33.9±0.51	32.6±0.39	36.4±0.51
Metacarpal, length	6.6±0.07	7.7±0.11	7.5±0.09	7.5±0.14
Pelvic girdle				
Length	29.3±0.77	47.7±1.20	40.9±1.12	46.3±1.18
Width, intercristal	15.8±0.54	28.7±0.81	26.7±0.89	35.5±0.80
Width, interacetabular	7.8±0.54	13.9±0.60	12.5±0.26	16.6±0.61
Width, intertuberous	10.8±0.14	18.3±0.45	13.5±0.05	17.7±0.43
Thickness of bone, ilium	1.1±0.54	2.5±0.12	2.1±0.08	2.9±0.01
Hind-limb				
Femur, length	24.0±0.46	35.2±0.52	33.8±0.44	39.7±0.76
Tibia, length	30.8±0.63	43.4±0.64	39.6±0.76	44.6±0.30
Tibia, width of head, ant.-post.	4.8±0.05	6.5±0.17	6.0±0.08	6.7±0.21
Tibia, width of shaft, lateral	2.0±0.04	2.8±0.16	2.8±0.10	3.6±0.07
Calcaneus, length	7.6±0.10	8.2±0.15	8.2±0.15	8.0±0.37

of the injected hypophysectomized rats which were equal to normal (i.e., did not significantly differ from normal) are as follows: total body length (also length from nose to anus and anus to tail-tip), lengths of the skull, cranium, thorax, clavicle, humerus, radius, ulna, metacarpal, femur and calcaneus, and width of pelvis (intercristal and interacetabular). The dimensions in which these rats significantly exceeded normal were width of thorax (by 9% at the level of the 1st rib, and 14% at the 10th to 11th rib), length of tibia (10%), width of cranium (14%), width of scapula (14%), thickness of lumbar vertebrae (14%), bizygomatic width of skull (17%), thickness of ilium (19%), length of scapula (27%), intertuberous width of pelvis (35%), and height of deltoid tuberosity (76%). The skeletal dimensions of the injected hypophysectomized rats therefore equalled or exceeded normal. Thus some differences in proportions between the hypophysectomized rat and adult normal were corrected, other were even exaggerated. The disproportion between metacarpal length and body length was corrected, that between length of tibia and body length was maintained, the disproportion between skull width and skull length was exaggerated.

Since in hypophysectomized rats injection of growth hormone resulted in growth in excess of normal in some dimensions the next step was to compare their response to growth hormone to that of normal animals. In intact rats, the prolonged administration of growth hormone had produced gigantism (Evans, Becks, Asling, Simpson, and Li, 1948). During this overgrowth, normal proportions had on the whole been maintained, most skeletal dimensions exceeding those of normal adults by 12-16%. The disproportions which were found were restricted to such bones as the metacarpal and calcaneus, where epiphyseal closure had occurred before the injections began.

The total body length of hypophysectomized injected rats was 14% less than that of the intact giants. The individual dimensions which fell short of those of intact giants were lengths of clavicle (20%), thorax (15%), cranium (14%), humerus (13%), femur (11%), skull (9%), and radius and ulna (8%), and widths of pelvis (intercristal, 19% and interacetabular, 16%), and scapula (12%). The injected hypophysectomized rats equalled (i.e., did not differ significantly from) injected normal rats in widths of skull, cranium, thorax (at both levels measured), and pelvis (intertuberous), and lengths of pelvis, tibia, calcaneus, and metacarpal, and thickness of

ilium. They exceeded the intact giants in length of scapula (11%) and height of deltoid tuberosity (28%). The injected hypophysectomized rats equalled or even exceeded the intact giants in some dimensions; however, in the majority of dimensions they failed to attain gigantism.

The foregoing analysis has dealt primarily with lengths and widths of the various skeletal components. However, in the case of complex structures such as the skull, thoracic cage, and pelvis, proportions are best determined by ratios between lengths and widths, and by the angular relationships between selected points of reference. Such relationships are shown in Figs. 5-8 and in Table 3.

TABLE III
MEASUREMENTS OF PROPORTIONS OF SKULL AND PELVIS OF HYPOPHYSECTOMIZED AND NORMAL RATS, UNINJECTED AND INJECTED CHRONICALLY WITH GROWTH HORMONE

	Hypophysectomized (age 480 days)		Normal (age 650 days)	
Ratio or Angle	Uninjected	Injected	Uninjected	Injected
SKULL				
Ratio of Maxima of skull*	52.4 ± 1.17	62.6 ± 1.69	53.2 ± 0.85	55.7 ± 0.94
Cranial Index*	79.2 ± 2.15	88.8 ± 3.43	71.8 ± 1.89	74.0 ± 1.85
Cranium-skull length ratio*	53.6 ± 0.93	52.2 ± 1.42	54.8 ± 2.06	52.9 ± 0.85
Zygo-nasal angle	42.2° ± 0.80°	50.8° ± 1.66°	39.7° ± 0.76°	41.0° ± 0.84°
Zygo-occipital angle	71.8° ± 0.86°	78.4° ± 1.29°	79.0° ± 2.18°	76.8° ± 0.95°
PELVIS				
Supra-pubic angle	56.0° ± 1.95°	80.0° ± 3.18°	72.5° ± 1.80°	88.3° ± 2.28°

*Ratio expressed as percent.

Figure 5 shows tracings of roentgenograms of representative skulls from the four groups of rats in the following order: Hypophysectomized, control (a), injected (b); Normal, control (c), injected (d). The first row, a_1 to d_1, shows their relative size; the second row, a_2 to d_2, shows the skulls drawn to the same length in order to make the differences in shape and proportion more evident. The dotted line indicates the cranium, a term herein restricted to the brain-case. The uninjected hypophysectomized rats had a cranial outline which was relatively rounder than that in the uninjected normal rats. In this respect they were like very young rats, in spite of their much greater age. The injections of growth hormone into hypophysectomized rats accentuated this characteristic. On the other hand, growth hormone did not appreciably alter the cranial contour of

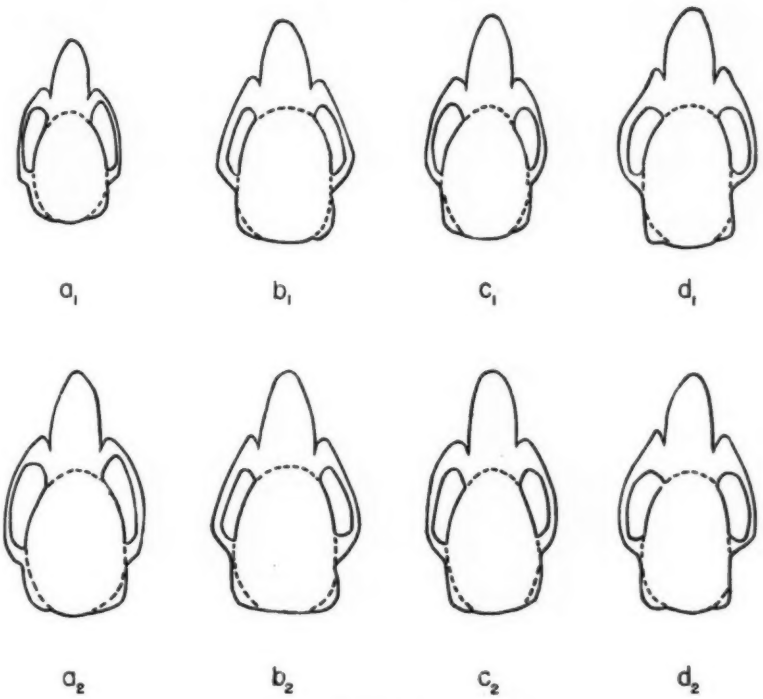

FIGURE 5

Tracings of dorso-ventral roentgenograms of representative skulls from the four groups of rats. (Dotted lines indicate the cranium.)

a. Hypophysectomized female rat 449 days after operation at 26 days of age.
b. Hypophysectomized female rat of similar age, injected with growth hormone for 449 days, beginning 2 weeks after operation.
c. Normal female rat 650 days of age.
d. Normal rat of similar age injected with growth hormone for 437 days.

(a_1 to d_1 tracings of actual size; a_2 to d_2 drawn to the same longitudinal base-line.)

intact rats. The non-cranial parts of the skull, both rostrally and laterally, were delicate and undeveloped in the hypophysectomized control. They became much broader in the injected hypophysectomized rat. In breadth of cranium, rostral portion of the skull and zygomatic arch these injected hypophysectomized rats surpassed the injected normal animals.

In the analysis of the proportions of the skull both linear and

angular measurements were made between the points indicated by the letters A to E in Fig 6. Linear measurements were as follows: skull length—rostral extremity of nasal bone (A) to caudal extremity of supraoccipital bone (B); bizygomatic width—between caudal ends of zygomatic arches (C to C′); cranial length—maximum length of cranium (B to D); and cranial width—maximum width of cranium (E to E′). The linear measurements have been recorded in Table 2. The ratios calculated from these linear measurements are given in Table 3. They were as follows: Ratio of bizygomatic width to skull

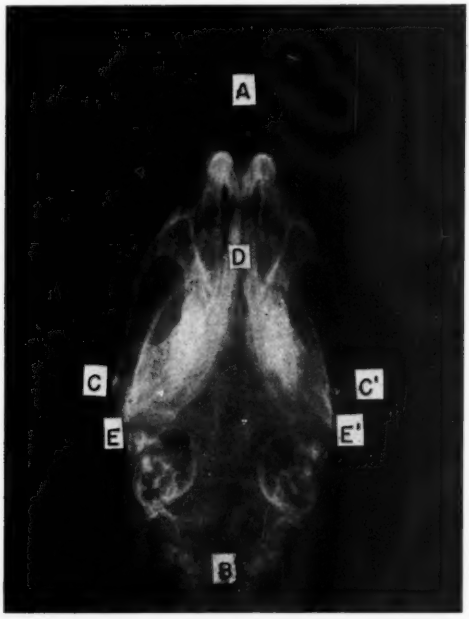

FIGURE 6

Dorso-ventral roentgenogram of the skull of a normal female rat 27 days of age (the age of operation in this experiment).

A. Rostral extremity of nasal bone.
B. Caudal extremity of supraoccipital bone.
C, C′. Caudal end of zygomatic arch.
D. Rostral extremity of cranium.
E, E′. Points marking greatest width of cranium.

length (ratio of maxima of skull); ratio of cranial width to length (cranial index); ratio of cranial length (cranium-skull length ratio). The angular measurements also appear in Table 3; they were as follows: zygonasal angle—the internal angle at the rostral end of the skull (CAC′); zygooccipital angle—the internal angle at the caudal end of the skull (CBC′).

The cranial index offers a measure of the relative roundness of the cranium. In the uninjected hypophysectomized rats this index (79.2) was that characteristic for young[5] normal rats (78.3) rather than the adult normal rats used as controls in this series (71.8). In intact rats injected with growth hormone the cranial index (74.0) did not differ significantly from the normal adult value. In injected hypophysectomized rats its value greatly increased (88.8).

The question arose whether the brachycephaly of the injected hypophysectomized rats was due to restricted growth in the longitudinal axis or excessive growth in the transverse axis. The longitudinal growth of the cranium, in relation to that of the whole skull, is represented by the cranium-skull length ratio (Table 3). This value was the same in all four groups of rats. Thus the longitudinal cranium-skull proportions of injected hypophysectomized rats were like those of normal adults, and the growth resulting from growth hormone injection was proportional.

In the transverse axis disproportionate growth could be shown in the injected hypophysectomized rats. The crania were broader than normal in absolute measurements. Their breadth even exceeded that of the injected intact rats (Fig. 5, b_1 to d_1). The bizygomatic dimension was also broader than normal in absolute measurement, as broad as the injected intact rats. The ratio of maxima of the skull was greatest in the injected hypophysectomized rats. The zygonasal angle

[5]Measurement of the roentgenograms of 10 female rats 27 days of age were made to determine some of the proportions of the skull in youth. The averages and standard errors of these measurements were as follows:

Skull length	33.0 ± 0.31 mm.
Bizygomatic width	18.6 ± 0.32 mm.
Cranial length	20.1 ± 0.12 mm.
Cranial width	15.7 ± 0.04 mm.
Zygo-nasal angle	46.0 ± 0.36°
Zygo-occipital angle	78.0 ± 0.68°
Ratio of maxima	56.1 ± 0.53
Cranial index	78.3 ± 0.43
Cranium-skull length ratio	60.5 ± 0.22

of normal rats (39.7°) was exceeded in the injected hypophysectomized rats (50.8°). The changes in head form resulting from his excessive increase in breadth were visible even during life, when the width and bluntness of the heads of these rats received special comment (Figure 2, Simpson, Evans, and Li, 1949).

Figure 7 illustrates the size and shape of the thorax of rats representative of the four groups. When drawn to the same length it is seen more clearly that there are no outstanding differences in shape. The main difference was in the position of the costochondral junction (shown in dotted lines on the diagrams). As previously stated, this junction was displaced laterally in the injected hypophysectomized rats (b_1, b_2).

The relative differences in size and shape of the pelvis are illustrated in Figure 8. In the uninjected hypophysectomized rats (a_1, a_2) the pelvis was essentially small, narrow and tubular. In injected hypophysectomized rats (b_1, b_2) the pelvis was much larger though still tubular. The rami of the ischia were slightly everted. The uninjected normal rats (c_1, c_2) had flared iliac crests and the cranial portion of the pelvis thus appeared funnel-shaped. The ischial rami were straight. The injected normal rats (d_1, d_2) showed even more flare of these crests, while the ischial rami curved inward giving the pelvic walls a sigmoid contour. This was accentuated by the thickened bone in the mid-iliac region, which encroached upon the pelvic cavity and distorted its tubular form. The tuberosities of the ischia appeared prominent in both groups of rats injected with growth hormone (Fig. 8, b_1 and d_1). It could not be determined from the roentgenograms whether this appearance resulted from the pelvic inclination or from bony accretion. Figure 8 also shows that the angle at the pubic symphysis, formed by the superior rami of the pubis, differed in the four groups of rats. In normal rats it measured 72.5°. The angle was less than normal in uninjected hypophysectomized rats (56.0°). The angle was increased in the injected hypophysectomized rats (80.0°), and was still greater in injected intact rats (88.3°).

Summary

The effects of the prolonged administration of growth hormone on the skeletons of hypophysectomized and normal rats have been compared by study of roentgenograms. The differences in response were evaluated from the general appearance of the skeleton, measurements

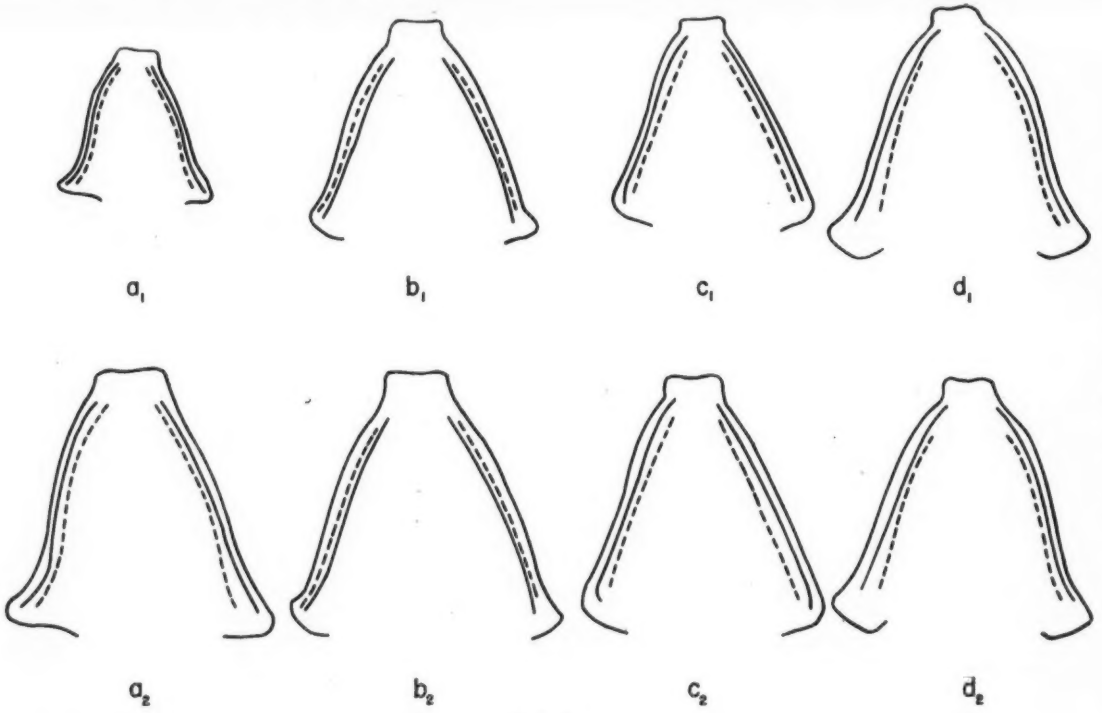

FIGURE 7

Tracings of dorso-ventral roentgenograms of the thorax of rats representative of the four groups. (The position of the costochondral junction is indicated by dotted lines.)

a. Hypophysectomized female rat 449 days after operation at 26 days of age.
b. Hypophysectomized rat of similar age injected with growth hormone for 437 days, beginning 2 weeks after operation.
c. Normal female rat 650 days of age.
d. Normal female rat of similar age, injected with growth hormone for 437 days.

(a_1 to d_1, tracings of actual size; a_2 to d_2 drawn to the same longitudinal base line.)

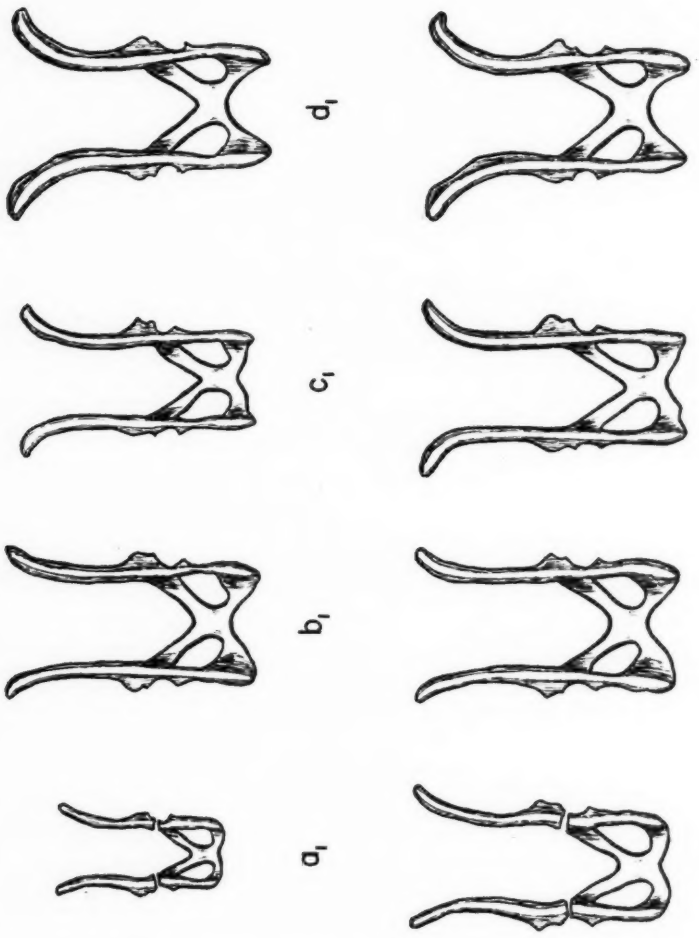

FIGURE 8

Tracings of dorso-ventral roentgenograms of the pelvis of rats representative of the four groups.

a. Hypophysectomized female rat 449 days after operation at 26 days of age.
b. Hypophysectomized female rat of similar age injected with growth hormone for 437 days beginning 2 weeks after operation.
c. Normal female rat 650 days of age.
d. Normal female rat of similar age, injected with growth hormone for 437 days.
a_2 to d_2 tracings, of actual size; a_1 to d_1 drawn to same longitudinal base-line.)

of various bones, status of the epiphyseal lines, and from indices and angles determined on the more complex skeletal structures (skull, thorax, and pelvis).

The injection of growth hormone caused growth in all parts of the skeleton measured, in both hypophysectomized and intact rats, with

the exception of the metacarpals and calcaneus of the intact rats, where epiphyseal fusion had occurred before the onset of injection.

The status of the epiphyseal lines in the injected hypophysectomized rats was brought to that characterizing normal rats. Injected intact rats did not show premature epiphyseal fusion.

In the injected hypophysectomized rats all bones reached normal length; they often exceeded normal and even equalled those of injected intact rats but rarely (length of scapula and height of deltoid tuberosity) exceeded those of intact rats similarly treated.

In the growth of the hypophysectomized rats injected with growth hormone many skeletal disproportions developed, notably in the head and pelvis. In the injected intact rats, however, proportionality was maintained in the skeletal growth, almost without exception.

c. Normal female rat 650 days of age.
d. Normal female rat of similar age, injected with growth hormone for 437 days.
(a, to d, tracings of actual size; a, to d, drawn to same longitudinal base-line.)

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THE GROWTH OF HYPOPHYSECTOMIZED FEMALE RATS FOLLOWING CHRONIC TREATMENT WITH PURE PITUITARY GROWTH HORMONE[1]

V. Skeletal Changes: Skull and Dentition

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An analysis of the skeletal changes in hypophysectomized rats treated chronically with pure growth hormone and a comparison of these changes with those found in similarly treated normal rats have been described in the two preceding papers (3, 6). Since in normal rats treated chronically with growth hormone severe distortions in the mandibular condyle and a marked elongation of the molar roots were found (2), the mandibular condyle, incisors, and molars of the rats of this series were again made the subject of study. The results are reported in this paper.

A detailed description of the experimental conditions was given in Paper I of this series (8) and may be summarized as follows: Female rats were hypophysectomized at 26-28 days of age. Ten of these survived the full experimental period and were sacrificed 449 to 462 days after operation. Their ages then were 475 to 488 days. Five of the rats were controls; the other five had received injections of pure pituitary growth hormone for 437 days starting 12 days after hypophysectomy. Five normal rats of the same age (462 to 595 days) were used for comparison. At the termination of the experimental period, the rats were sacrificed and the heads prepared for histologic study. They were first separated sagitally into halves, roentgeno-

[1]Aided by grants from the American Cancer Society, Inc., Research Fund, New York City, the United States Public Health Service (RG-409), (RG-800R), Washington, D. C., the Board of Research of the University of California, the Rockefeller Foundation, New York City, The American Foundation for Dental Science and the California State Dental Association.

graphed and then decalcified. The mandibular condyle, teeth and fronto-squamosal suture were sectioned and stained with hematoxylin and eosin.

Skull, Maxilla and Mandible

A skull roentgenogram of a normal rat is shown in Fig. 1. The bones of the head are well calcified and the molars are normal in outline, with straight roots. The incisors also have a normal appearance, with apices open and pulp chambers extending well into the middle half of the tooth.

The effects of hypophysectomy on the skull of the rat are shown in Fig. 2 and correspond to those previously reported (Becks, Collins, Simpson, Evans, 1946). The outstanding differences from the normal are severe dwarfism and changes in the growth and development of the teeth. The molars show little change from the normal except for slightly smaller size. The incisors are distorted in outline in four of the five uninjected hypophysectomized rats, with irregular folds occurring at the apical third. The pulp chamber is greatly reduced in size due to continued formation of dentin associated with arrested tooth eruption.

The skull of a hypophysectomized rat which received chronic injections of growth hormone is shown in Fig. 3. The skull itself and all bones appear larger and more densely calcified than those of the normal of the same age. The central incisors are larger than normal; the pulp cavity is open at the apex, but three-fourths of the pulp cavity has been obliterated by the deposition of dentin. Apical folding, tyical for hypophysectomized rats after long post operative periods (1), was not seen; however, it can be observed that the layer of enamel on the labial side is extremely thin and has a rough surface. A very striking alteration in the development of both the upper and lower incisors was the acute curvature.

The primary objective of the roentgenographic study was to compare the longitudinal and vertical growth of the lower and upper jaws. The points of measurement are shown in Figure 4 and were defined as follows: reference points for longitudinal dimensions in the lower jaw were the posterior border of the mandibular condyle (A); the posterior margin of the angle of the mandible (Y); the mesial surface of the lower 1st molar (B); a point on the enamel surface of the apical third of the lower incisor direct in line between

ROENTGENOGRAMS OF SAGITTAL SECTIONS OF THE SKULL OF FEMALE RATS (Dotted lines indicate the outline of the mandibular condyle) × 2

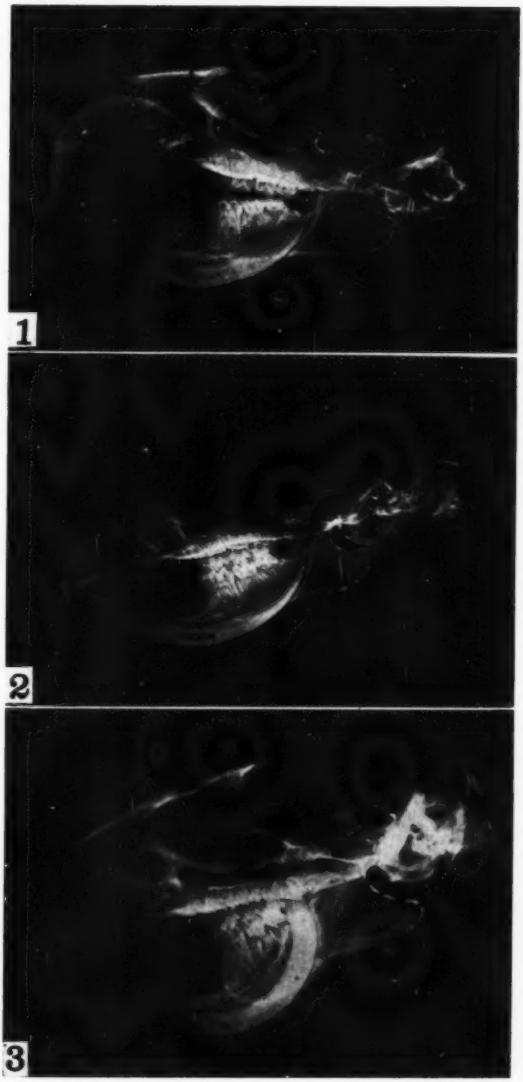


FIGURE 1
Normal rat, 449 days of age. Sp. 6761, Pl. B378.

FIGURE 2
Hypophysectomized rat, 449 days after the operation at 26 days of age. Sp. 9020, Pl. B379.

FIGURE 3
Hypophysectomized rat injected with growth hormone for 437 days, beginning 12 days after the operation at 26 days of age. Sp. 9024, Pl. B380.

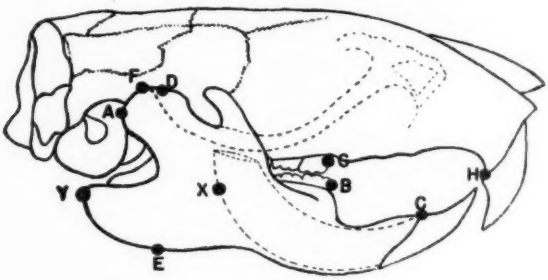


FIGURE 4

DIAGRAM OF RAT SKULL, SHOWING REFERENCE POINTS FOR MEASUREMENTS

A. Posterior border of the mandibular condyle
Y. Posterior margin of the angle of the mandible
B. Mesial surface of the lower 1st molar
X. A point on the enamel surface of the apical third of the lower incisor in a direct line between Y and B
C. Lingual alveolar crest of the mandible
D. Superior surface of the mandibular condyle
E. Inferior border of the ramus directly opposite D.
F. Posterior border of the zygoma
G. Mesial surface of the upper 1st molar
H. Lingual alveolar crest of the maxilla

Y and B (X); and the lingual alveolar crest of the mandible (C). The reference points for determination of the vertical dimension of the mandible were the superior surface of the condyle (D); and the inferior border of the ramus vertically opposite (E). In the upper jaw, the reference points for longitudinal dimension were as follows: The posterior border of the zygoma (F); the mesial surface of the upper 1st molar (G); and the lingual alveolar crest of the maxilla (H).

In the injected rats, both upper and lower jaws grew, as may be seen in Table I by comparing their dimensions with those of the hypophysectomized controls. They even attained a size greater than in the normal rats, both in longitudinal and vertical dimensions. The lower jaw grew more in longitudinal dimensions (A-B) than did the equivalent segment of the upper jaw (F-G). The excessive growth of the mandible involved not only the condyle but also the angle of the mandible and the dental segments. The dimension Y-X shows the caudal extension of the angle of the mandible. Growth in the dental segment (containing the molar teeth and the apical portion of the incisor) is shown by the increase in the dimension X-B. The increase

TABLE I

COMPARISON OF LONGITUDINAL AND VERTICAL MEASUREMENTS OF THE LOWER AND UPPER JAWS OF NORMAL, HYPOPHYSECTOMIZED AND CHRONICALLY INJECTED HYPOPHYSECTOMIZED FEMALE RATS

	Measurements	Reference Points	Normal Control	Hypophysectomized Control	Hypophysectomized Injected
			mm.	mm.	mm.
Lower Jaw	Longitudinal	Posterior border of condyle to mesial surface of 1st lower molar (A — B)	17.7 ±0.35*	15.2 ±0.15	20.1 ±0.48
		Posterior border of condyle to lingual alveolar crest of mandible (A — C)	24.1 ±0.48	20.6 ±0.25	25.6 ±0.10
		Posterior extremity of mandible to posterior enamel surface of lower incisor (Y — X)	4.2 ±0.33	5.7 ±0.13	9.7 ±0.24
		Posterior enamel surface of lower incisor to mesial surface of lower 1st molar (X — B)	9.4 ±0.70	9.5 ±0.17	10.2 ±0.25
	Vertical	Superior surface of condyle to inferior border of ramus of mandible (D — E)	11.9 ±0.26	9.4 ±0.17	13.2 ±0.53
Upper Jaw	Longitudinal	Posterior border of zygoma to mesial surface of upper 1st molar (F — G)	16.4 ±0.50	13.7 ±0.21	17.1 ±0.18
		Posterior border of zygoma to lingual alveolar crest of maxillae (F — H)	28.4 ±0.67	24.4 ±0.20	30.5 ±0.50

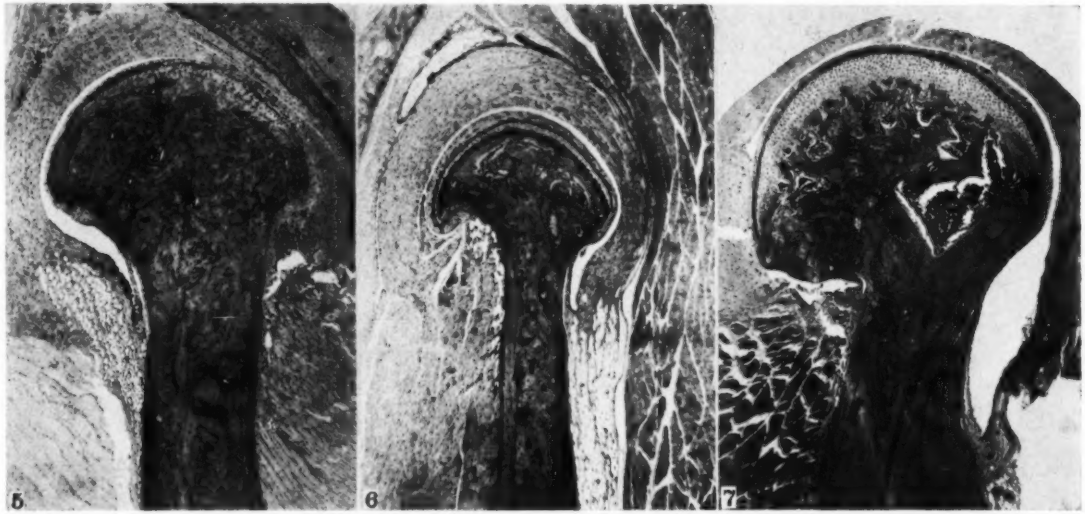
*The figures preceded by ± are the standard errors.

in the vertical dimension of the lower jaw (D-E) is also very marked.

The condylar heads of the mandible were studied histologically. Several features are distinctive for this bone (4). No secondary center of ossification develops. Cartilage present under the fibrous covering of the condyle remains as the growth center. The ossification of the entire head and ramus of the condyle is completed at approximately 250 days of age. A photomicrograph of a mandibular condyle of a normal rat 449 days old is shown in Fig. 5. It can be seen that the bone is extremely compact. Only a few osteoblasts were recognizable lining the small marrow cavities. The junction of the osseous part and the uncalcified cartilaginous zone of the condylar head can be clearly seen.

Hypophysectomy accelerates the ossification of the condylar head and ramus (5); by 28 days after the operation the condition is comparable to that of the normal 250-day old rat. No further changes occur in older hypophysectomized rats. Fig. 6 shows a photomicrograph of the condyle of a hypophysectomized untreated rat 449 days after operation. The very much smaller size of the condyle is noteworthy, especially if compared with that of the hypophysectomized injected rat (Fig. 7). A sharp basophilic zone marks the junction between the cartilaginous and osseous portion of the condyle; otherwise it is a miniature replica of a normal condyle of the same age (Fig. 5).

The condyles of the five hypophysectomized injected rats were remarkably similar. One representative photomicrograph is shown in Fig. 7. They are all outstandingly increased in size; in fact, their diameter is approximately 1/3 larger than normal. A wide zone of uncalcified embryonic cartilage is present and erosion is still occurring in some areas. Many of the histological features were those which characterize a normal rat of 150 days of age, when slow growth still occurs. The ramus is well ossified, but in contrast to the normal control (Fig. 5) a large number of marrow cavities are present. The mandibles of the hypophysectomized growth hormone treated rats differed, therefore, markedly from those of normal chronically treated animals injected for a comparable period (437 days) (2). In the latter, considerable distortion of the mandibles occurred, the degree of distortion differing greatly between individual animals.



SECTIONS THROUGH MANDIBULAR JOINT OF FEMALE RATS. H. and E. × 33

FIGURE 5
Normal rat 449 days of age. Sp. 8367, Pl. 9191.

FIGURE 6
Hypophysectomized rat 449 days after the operation at 26 days of age. Sp. 9017, Pl. 9871.

FIGURE 7
Hypophysectomized rat injected with growth hormone for 437 days, beginning 12 days after the operation at 28 days of age. Sp. 9025, Pl. 9875.

Teeth

A photomicrograph of the upper incisor of a normal rat 458 days of age is shown in Fig. 8. The outline of the tooth, on both the inferior and superior surface is smooth. The pulp chamber, with its rich blood supply, occupies the greater portion of the apical half of the tooth. It becomes smaller in the middle third and is finally reduced to a fine opening at the incisal edge. At the apex the dentin and enamel begin as a fine line and gradually increase in thickness toward the incisal surface. From the center of the tooth to the incisal surface the canal is only about 20 μ in diameter and accommodates atrophic pulp. The dentin shows normal calcification. Under high magnification fine incremental lines are discernible, which were described by Schour *et al.* (7), as indicative of the daily calcification rhythm. In the apical third, where dentinogenesis is most active, the layer of pre-dentin is approximately 40 μ wide on the labial side and 20 μ on the lingual side. The odontoblasts are active throughout the apical third and are present in the middle third. The enamel epithelium has a normal appearance throughout the apical two-thirds, but is reduced to a thin line of flattened cells in the incisal third. The enamel has been lost during the decalcification process. Its width is indicated by the empty space next to the dentin on the labial side.

The changes in the incisors of the hypophysectomized controls corresponded closely to those previously described as characteristic for animals following long postoperative periods (1). In 4 out of 5 the apical third showed severe distortions (Fig. 9). The teeth were smaller and the pulp canal was almost completely obliterated through continued deposition of dentin. The pulp canal showed only a slight opening at the apical end. Odontoblasts and ameloblasts showed severe degenerative changes.

The incisors of the growth hormone injected hypophysectomized rats were approximately normal in size, but showed several structural differences from normal (Fig. 10). Although there was no "accordion-like" folding of the dental tissues at the apical end, typical of the uninjected hypophysectomized rats of this age, the superior surface presented irregular elevations and depressions; these were present over the entire enamel surface. (The changes at the apical end corresponded to the degree of distortion observed in hypophysectomized

SECTIONS THROUGH UPPER INCISOR OF RATS.
H. and E. × 5.5

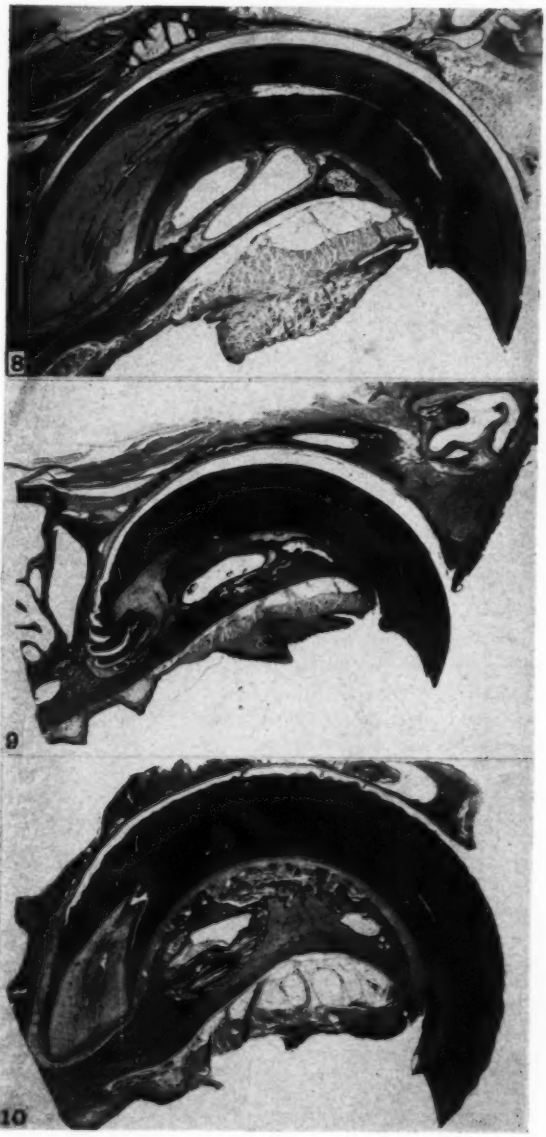


FIGURE 8
Normal rat 458 days of age. Sp. 8364, Pl. B349.

FIGURE 9
Hypophysectomized rat 449 days after the operation at 26 days of age. Sp. 9020, Pl. B372.

FIGURE 10
Hypophysectomized rat injected with growth hormone for 437 days beginning 12 days after the operation at 26 days of age. Sp. 9024, Pl. B376.

rats as described by Becks *et al.* (1). The layers of dentin were very thick, almost obliterating three-fourths of the pulp canal. The odontoblasts were only present in the apical fourth. Incremental lines were distinct and measured approximately 10μ apart. Since in the normal deposition of dentin they are 16μ apart (Schour), this indicates that growth was not restored to normal.[2] The enamel epithelium was reduced to a very fine line in every hypophysectomized injected rat. In three out of five, amelogenesis had ceased completely in the apical fourth leaving dentin in direct contact with the connective tissue. Higher magnifications of the apical thirds are shown in Figures 11, 12 and 13.

In the molars no significant changes were found except for a slight increase in cementum formation at the apical end of the roots. The increase, however, was not as marked as in the intact giants.

Calvarium

As previously reported in normal rats, the injections of growth hormone stimulated intramembranous osteogenesis in the skull. The calvarium of the rats injected with growth hormone was markedly thicker than in the uninjected hypophysectomized controls. The calvarium of the uninjected rats was thin, and at autopsy seemed brittle and easily splintered. Fig. 14 shows a coronal section through the fronto-squamosal suture of an uninjected hypophysectomized rat, and Fig. 15 shows its counterpart in a rat chronically injected with growth hormone. The difference in thickness of bone is apparent. In the hypophysectomized control, the connective tissue between the two bones is scanty and the bones are closely approximated. Synostosis, however, has not occurred. Marrow spaces can be seen within the bones. The lack of active bony accretion is shown by the uniformity of staining, lines of increment of bone being few and widely separated. In the injected rat, the connective tissue of the suture is increased in amount. The bone is dense and without marrow islands. Bone deposition has been active, as demonstrated by the multiple lines of bony accretion. It would appear from the distribution of the lines of accretion that bone resorption was occurring on one side and deposition on the other side of the suture.

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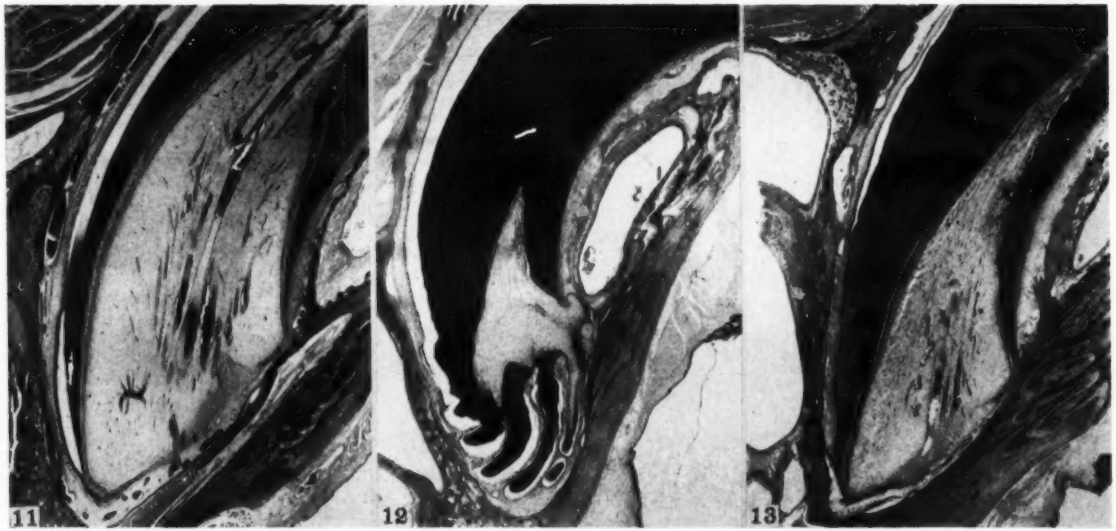
[2]The eruption rate of the upper incisors was measured over a period of 5 weeks during the middle of the experiment and measured 1.21 mm. per week as compared with 2.4 mm. for the normal, as well as for the intact giants.

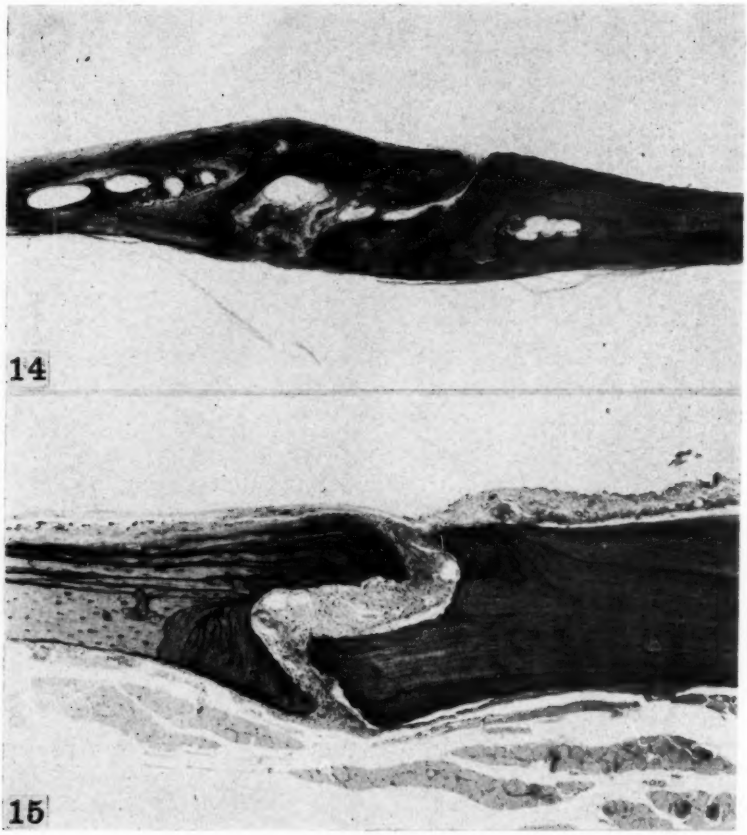


HIGHER MAGNIFICATION (× 11) OF THE APICAL THIRDS OF THE INCISORS SHOWN IN FIGURES 8, 9, 10

FIGURE 11
Normal rat. Pl. B375.

FIGURE 12
Hypophysectomized rat. Pl. B373.

FIGURE 13
Growth hormone treated hypophysectomized rat. Pl. 374.



CORONAL SECTIONS THROUGH THE FRONTO-SQUAMOSAL SUTURE OF HYPOPHYSECTOMIZED RATS 449 DAYS AFTER THE OPERATION AT 26 DAYS OF AGE. H. and E. × 100

FIGURE 14
Uninjected control. Sp. 9020, Pl. B356.

FIGURE 15
Growth hormone injections for 437 days beginning 12 days after the operation at 26 days of age. Sp. 9024, Pl.. B355.

Summary

The effect of chronic administration of growth hormone on the skulls of hypophysectomized animals was subjected to a careful analysis. The jaws, teeth, and calvarium were studied. A general increase in size of upper and lower jaws over their normal and hypophysectomized controls was found. The increase of the lower jaws was greater than that of the uppers. The growth of a posterior portion of the mandible contributed much to the increase in longitudinal and vertical dimensions. The angle of the mandible showed a marked flaring which was found in all injected animals.

Histologically the mandibular condyles were greatly enlarged but otherwise normal in shape. At the termination of the experiment, 437 days after operation, there was still active chondrogenesis and erosion and the structure was comparable to that of a normal 150 day old rat.

The incisors were larger than their hypophysectomized controls, but not quite as large as their normal controls. The curvatures of both the upper and lower incisors were more acute. Irregularities in outline of the labial surface of the enamel resulted from degenerative changes in the enamel epithelium. Amelogenesis had ceased in the apical third, leaving the dentin surface in direct contact with the peridental membrane. The rate of eruption was reduced to half of that observed in normal controls resulting in a slight distortion of the apical end. The pulp canal was obliterated by continued deposition of dentin with the exception of a small opening at the apex.

The calvarium of the treated animals was thicker and showed a large number of lines of accretion.

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Growth, 1949, **13**, 221-267.

SKELETAL GROWTH IN ANCON SHEEP

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As reviewed elsewhere, the Ancon mutation in sheep is inherited as a recessive character which, in homozygotes, causes disproportionate development. It has had two independent origins. The first occurred in Massachusetts in the late part of the eighteenth century. The name "Ancon" was used because of the bent legs of the affected animals (5). These Ancon sheep became extinct in the late nineteenth century.

The second shortlegged mutation appeared in 1919 in eastern Norway. Wriedt (16) studied the inheritance of this new mutation and also used the name "Ancon" for it, mainly because the two mutations are similar in body conformation, crippling and shortening of legs.

Ancon sheep are chondrodystrophic or achondroplastic. The literature on human chondrodystrophy is tremendous and has been reviewed from time to time (2, 11). As will be mentioned elsewhere, hereditary disproportionate dwarfism has also been known in a variety of animals. The genetic and pathological studies in some of them are not at all adequate. However, there is evidence that not all chondrodystrophic mutations are identical. The most thoroughly investigated case of chondrodystrophy is the Creeper fowl (8, 9, 10). Since the genetical analysis of Wriedt there have been no further reports on Ancon sheep. The present report contains data derived from a study of the skeletal growth of Ancon sheep originated from the Norwegian mutation.

MATERIAL AND METHODS

Material

This Department obtained two heterozygous ewes and a homozygous one from Wriedt in 1927. Since then a small flock has been raised and maintained by inbreeding and occasional outcrossing to Cheviots. Fifty homozygous (Ancon) and fourteen heterozygous (normal) skeletons were accumulated over a period of years and provided the material for the present study. These skeletons consist of newborn, 2-month, 4-

month, 6-month and adult stages. Due to insufficient material for more classes, those above one year of age were all included in the adult stage, two 2½-month old Ancons were counted as 2-month and two 3½-month old Ancons as 4-months. There were only males in the 4-month and 6-month stages of both Ancon and normal sheep and only females among the adult normals.

The appendicular bones of all ages and axial skeletons and pelves of sheep younger than two months were preserved in 10% formalin. All the rest were preserved dry. The distribution of material in relation to age and sex is shown in Table I. From this table one will see that the number of leg bones does not correspond to the number of individuals because, in a few cases, only one of the paired legs was preserved. In still others the digits may be incomplete.

The distribution of individuals in relation to twinning in our material was as follows. Within each age group the first number gives the animals from single births, the second one from twin births, and the third that from triplets.

	Normal		Ancon	
	male	female	male	female
Newborn	2-1-0	1-0-0	8-4-0	8-7-0
2-month	0-1-0	0-1-0	2-0-0	1-0-1
4-month	1-1-0		3-3-0	
6-month	1-0-0		2-3-0	
Adult		5-0-0	4-1-0	10-0-0

Measurements. The formalin preserved bones were cleaned as much as possible, especially in places that were to be measured. Structures with clear demarkation were chosen and measured with sliding calipers.

The following measurements were obtained:

A. Skull

I. Length

1. SkL—total skull length, from the supra-occipital spine to the tip of the premaxilla.
2. BSk—length of the skull base, from the intercondyloid incisure to the tip of the premaxilla.
3. PL—palatal length, from the posterior edge of the interpalatine suture to the tip of the premaxilla.

II. Width

4. PmW—premaxillary width at the level of bifurcation.
5. IFT—distance between the two interfacial tuberosities.

TABLE I

SHOWING THE NUMBER OF INDIVIDUALS AND BONES (IN BRACKETS) IN DIFFERENT AGES OF NORMAL AND ANCON MALES AND FEMALES

	Newborn				2 months				4 months		6 months		Adult			Total	
	Normal	Ancon	Normal	Ancon	Normal	Ancon	Normal	Ancon	Normal	Ancon	Normal	Ancon	Ancon	Normal	Ancon	Normal	Ancon
	♂		♀		♂		♀		♂		♂		♂	♀			
Foreleg																	
Scapular	(6)	(22)	(2)	(15)	(2)	(2)	(2)	(4)	(4)	(12)	(2)	(6)	(8)	(10)	(17)		
	3	12	1	8	1	1	1	2	2	6	1	3	4	5	10	14	46
Humerus	(6)	(23)	(2)	(15)	(2)	(4)	(2)	(4)	(4)	(12)	(2)	(10)	(10)	(10)	(17)		
	3	12	1	8	1	2	1	2	2	6	1	5	5	5	10	14	50
Radius ulna	(6)	(22)	(2)	(15)	(2)	(4)	(2)	(4)	(4)	(12)	(2)	(10)	(10)	(10)	(17)		
	3	12	1	8	1	2	1	2	2	6	1	5	5	5	10	14	50
Metacarpus	(6)	(23)	(2)	(15)	(2)	(4)	(2)	(4)	(4)	(12)	(2)	(10)	(10)	(10)	(17)		
	3	12	1	8	1	2	1	2	2	6	1	5	5	5	10	14	50
1st phalanges	(12)	(44)	(4)	(30)	(4)	(8)	(4)	(8)	(8)	(20)	(4)	(18)	(20)	(20)	(34)		
	3	12	1	8	1	2	1	2	2	5	1	5	5	5	10	14	49
2d phalanges	(8)	(40)	(4)	(30)	(4)	(8)	(4)	(8)	(8)	(16)	(2)	(14)	(16)	(20)	(32)		
	2	11	1	8	1	2	1	2	2	4	1	4	4	5	10	13	45
Hindleg																	
Pelvis	2	9	1	6	1		1	1	2		1		1	5	8	11	25
Femur	(6)	(23)	(2)	(15)	(2)	(4)	(2)	(4)	(4)	(12)	(2)	(10)	(10)	(10)	(17)		
	3	12	1	8	1	2	1	2	2	6	1	5	5	5		14	50
Tibia	(6)	(23)	(2)	(15)	(2)	(4)	(2)	(4)	(4)	(12)	(2)	(10)	(10)	(10)	(17)		
	3	12	1	8	1	2	1	2	2	6	1	5	5	5	10	14	50
Metatarsus	(6)	(23)	(2)	(15)	(2)	(4)	(2)	(3)	(4)	(12)	(2)	(10)	(10)	(10)	(17)		
	3	12	1	8	1	2	1	2	2	6	1	5	5	5	10	14	50
1st phalanges	(12)	(44)	(4)	(30)	(4)	(8)	(4)	(6)	(8)	(20)	(4)	(18)	(20)	(20)	(34)		
	3	12	1	8	1	2	1	2	2	5	1	5	5	5	10	14	49
2d phalanges	(8)	(40)	(4)	(30)	(4)	(8)	(4)	(6)	(8)	(14)	(2)	(16)	(18)	(16)	(30)		
	2	11	1	8	1	2	1	2	2	4	1	5	5	4	10	12	47

TABLE I (*continued*)

	Newborn				2 months				4 months		6 months		Adult			Total	
	Normal	Ancon	Normal	Ancon	Normal	Ancon	Normal	Ancon	Normal	Ancon	Normal	Ancon	Ancon	Normal	Ancon	Normal	Ancon
	♂		♀		♂		♀		♂		♀		♂	♂			
Skull	3	12	1	8	1	1	1	2			2	4	3	5	8	13	38
Mandible	3	12	1	8	1	1	1	2			2	4	3	5	8	13	38
Vertebrae (complete set)																	
Cervical	1	3	1	3	1		1	1			1			2	2	7	9
Thoracic	1	3	1	3	1		1	1						1	2	4	9
Lumbar	1	3	1	3	1		1	1						1	2	5	9
Sacrum	1	4	1	3	1		1	1			2	1	1	2	7	8	17
Ribs (complete set)	1	2	1	3			1							1	2	4	7
Thoracic cavity	2	4	1	5	1	2	1	1	1				1	2	1	7	15

6. PtOW—supra- or posterior-orbital width, between the superio-posterior processes.
7. ZW—interzygomatic width, at the suture of the zygomatic processes of the malar and the temporal bones.
8. CW—cranial width, between the anterior medial wall of the temporal fossa marked by the fronto-orbital suture.

III. Height

9. CH—cranial height, from the basi-occipital-sphenoid suture to the fronto-parietal suture.
10. SKH—midskull height, from the vertex of the skull to the tip of the pterygoid process.
11. FH—facial height, from the inter-naso-frontal suture to the inter-maxillo-palatine suture.

IV. Occipital region

12. ICI—width of the intercondyloid incisure, distance between the medio-ventral margin of the two lateral condyles.
13. DCW—dorsal distance between the medio-dorsal margin of the two lateral condyles.
14. SOFM—distance between the squamous occipital and the foramen magnum, from the median parieto-squamous occipital suture to the dorsal lip of the foramen magnum.
15. SLFM—distance between the junction of the squamous lateral occipital suture to the dorsal lip of the foramen magnum.
16. OcSp—distance between the ventral lip of the foramen magnum and the occipito-sphenoid suture.
17. IPM—distance between the paramastoid processes of the occipital bones.
18. BOW—width of the basi-occipital bone.

V. Mandible

19. TL—total length of the mandible from the base of the incisor to the posterior margin of the angle.
20. CSL—condyle-symphyseal length, from the base of the incisor to the posterior margin of the condyle.
21. H—height of the mandible, from the coronoid to the base.
22. ICW—intercondyloid width.
23. IorW—intercoronoid width.
24. IAW—interangular width.
25. IMW—width of pars incisor mandibulae, distance between the lateral margin of the incisor.
26. WM—width at the level of the third molar (or the last cheek tooth).
27. TM—thickness at the level of the third molar (or the last cheek tooth).

B. Vertebrae

1. Height—from anterio-ventral surface of the centrum to the tip of the spinous process.
2. Width—between two transverse processes.
3. Length—ventral length of the centrum, dens included in the case of the axis.
4. Thickness—anterior thickness of the centrum.

In the case of the sacrum, the height, width, and thickness are the respective parts of S1, but the length is the total length of the sacral vertebrae.

C. Ribs

1. Length—from the costal tuberosity to the costochondral junction.
2. Width—the widest part of the rib, usually, in adult, 2-3 cm, and, in lambs, a a few mm. from the costochondral junction.

D. Thoracic cavity

1. Depth of the thoracic cavity—dorso-ventral diameter, from the ventral border of the centrum of T_5 to the inner surface of the sternum at the level of the 5th rib.
2. Width of the thoracic cavity—transverse diameter, widest horizontal distance between the inner surface of the 5th rib.

E. Extremities—The circumference of the leg bones was measured with very fine wire but the results were not used because the percentages of increase and retardation obtained from these data were not essentially different from those calculated from the diameters.

I. Forelimbs

1. Scapula
   a. Length—from the posterior border of the glenoid cavity to the termination of the scapular spine at the bone-cartilage junction.
   b. Width—distance between the anterior and posterior angles.
2. Humerus
   a. Length—the longest length of the bone. In order to see whether the great tuberosity or the head and neck increase more in the course of growth, there were two kinds of measurements: (a) the distance between the lateral condyle and the great tuberosity; and (b) the distance between the lateral condyle and the head.
   b. Diameter—diameter at the middle of the shaft. (This was taken at the same location in all long bones.)
3. Radius-ulna, length—from the proximal surface of the olecranon to the tip of the medial tuberosity of the radius.
4. Metacarpus, length—from the ridge between the two proximal articular faces to the medial and the lateral ridge of the respective distal articular surfaces as medial and lateral length.
5. First phalanx—longest length.
6. Second phalanx—longest length.

II. Hindlimb

1. Pelvis
   a. Length—anterior border of the tuber coxae to the posterior process of the tuber ischii.
   b. Width—anterio-ventral end of the symphyseal ridge to the highest ischiatic spine.
   c. Tuber coxae distance.
   d. Conjugate diameter.
   e. Transverse diameter.
   f. Distance between the dorsal processes of the tuber ischii.
   g. Symphyseal length.
2. Femur length—from the head or great trochanter to the trochlea or condyloid ridge.
3. Tibia length—from the intercondyloid eminence (spine) to the tip of the medial maleolus.
4. Metatarsus—the same as the metacarpus.
5. First phalanx—the longest length.
6. Second phalanx—the longest length.

Calculations. Owing to the wide range of individual variation in the Ancon sheep and the small number of the specimens available, only averages are used in this study. In order to save space the individual measurements and indices are not given in this report. They will be kept in the department for those who may be interested. The total number of cases is given in Table I and not repeated in the others. For calculation of averages, each individual is taken as a unit, that is

each unit is actually an average of the left and the right sides if it is a paired structure. However, in order to show the difference between the medial and lateral phalanges, each bone of the latter is taken as a unit in the calculation of the averages for length, diameter, and indices of diameter/length.

The figures for each kind of vertebra such as cervical, thoracic, and lumbar, are the average for all of one type in a given animal, that is the average of C_1-7, T_1-12 or 13, or L_1-6 or 7.

The length and width of the ribs are the average of all ribs.

The percentage increase is calculated according to the following formula:

$$\frac{V_2 - V_1}{V_1} \times 100$$

where V_1 is the size of newborn lamb and V_2 is that of a later stage of the same breed, so that the percentage given is the absolute percentage of increase with the size of the newborn as unity.

The percentage retardation is calculated by the same formula, but here V_1 is the size of the normal sheep and V_2 the size of the corresponding stage and sex of the Ancon. Since the size in some of the Ancon bones is larger than that of the normal while others are smaller, the plus and the minus signs are used to distinguish this difference.

With the exception of normal adults (which are lacking) the male material has more intermediate stages than the female. For the purpose of comparison the hypothetical size for the leg bones of the adult normal male has been calculated from the actual size of the adult Ancon male and female and adult normal female. This is based upon the assumption that the sex difference in the size of the normal and Ancon skeleton is proportional. From this hypothetical size the percentage increase for the normal male is derived. Figures based on such hypothetical values are placed in brackets in the tables to distinguish them from those based on actual measurements.

Results

A. Skull

Except for occasional abnormalities found in the occipital region, which will be described elsewhere, the skull in general is normal in appearance. Both the closure of sutures and the tooth formula failed to show any remarkable deviations. More extensive measurements on

the parts with clear demarkations were then used to detect any difference from the normal. The results are presented in Tables II and III. The following observations can be made from these measurements:

1. *Skull in general.* In Ancon sheep the length of the whole skull and of the occipital region and the width of the facial region are retarded, but the height of the skull, width of cranium and of the occipital bones are practically normal. The skull of the Ancon sheep is actually higher than normal in early stages but normal in the adult stage. Its percentage increase is consequently lowered. This indicates a higher prenatal and early postnatal growth rate in the skull height of Ancons. On the contrary, cases in which the percentage increase is greater in the Ancon but the actual size reached by the adult is smaller than the normal, as in the width of the facial region, are taken to indicate a higher relative retardation in the newborn, due to slower prenatal development.

II. *Mandible.* Concomitantly the mandible has the same type of retardation in length, height, and width as does the skull. The thickness of the mandible at the third molar region has a higher retardation than the rest of the structures measured. The mandibular bone then is normal in width, but reduced in length and thickness.

TABLE II

MEASUREMENTS (IN MM.) AND PERCENTAGE INCREASE OF NORMAL SKULL

	Age	Newborn		2 months		6 months	Adult
		♂	♀	♂	♀	♂	♀
a. Length							
1. SkL	average	120.6	118.4	131.2	148	199.6	232.7
	% increase			8.79	25	65.51	96.54
2. BSK	average	104.8	102	115.2	131	177.25	209.66
	% increase			9.9	25	69.13	105.54
3. PL	average	56.8	55.8	65.4	74.5	102.25	128.8
	% increase			15.14	33.51	80.02	130.82
b. Width							
4. PmW	average	13.1	14	12.7	12.3	21	28.02
	% increase					60.31	100.14
5. IFT	average	44.6	43.8	47.5	49.5	64	77.44
	% increase			6.5	13.01	43.49	76.80
6. PtOW	average	69.3	70.3	81.2	88.5	108.65	126.18
	% increase			17.17	25.89	56.78	79.48
7. ZW	average	61.4	62	68	72	95.75	111.2
	% increase			10.74	16.13	55.94	79.35
8. CW	average	53.5	53.6	57.4	61.2	70.5	66.02
	% increase			7.29	14.18	37.78	23.40

TABLE II (*continued*)
MEASUREMENTS (IN MM.) AND PERCENTAGE INCREASE OF NORMAL SKULL

	Age	Newborn ♂	Newborn ♀	2 months ♂	2 months ♀	6 months ♂	Adult ♀
c. Height							
9. CH	average	47.1	46	48	51.2	66.75	68.84
	% increase			1.91	11.30	41.72	49.65
10. SKH	average	55.3	48.7	58	63.5	91.4	100.1
	% increase			4.88	30.39	65.28	105.33
11. FH	average	29.7	30.8	36	39.5	57.5	68.36
	% increase			21.21	28.25	93.6	121.95
d. Occipital Region							
12. ICI	average	5.8	7.3	5.4	8	5.85	8.2
	% increase				9.58	0.86	12.33
13. DCW	average	17.5	18.4	16	20.5	20.25	22.3
	% increase				11.41	15.71	21.19
14. SOFM	average	26.1	26.5	30	31	46.7	40.84
	% increase			14.94	16.98	78.92	54.11
15. SLFM	average	3.5	2.6	6	5.7	13.9	
	% increase			71.41	119.23	297.14	
15. OcSp	average	16.8	15.7	17.5	19.2	26.2	25.92
	% increase			4.17	22.29	55.95	65.09
17. IPMN	average	44.25	40.4	46.5	50	62.65	68.64
	% increase			5.08	23.76	41.58	69.9
18. BOW	average	14.9	14.8	16.5	19.3	24.3	26.9
	% increase			10.73	30.41	63.08	81.75
e. Mandible							
19. TL	average	82.8	85	105	113	150.55	184.62
	% increase			26.81	32.94	81.82	117.20
20. CSL	average	91.5	94.5	110.6	119.5	165.25	197.18
	% increase			20.87	26.46	80.60	108.65
21. H	average	38.7	41.9	55.4	59	90.9	109.04
	% increase			43.15	40.81	134.88	160.24
22. ICW	average	54	54	52	58	68.25	80.86
	% increase				7.4	26.39	49.74
23. IorW	average	56.4	59.5	57.5	64.5	67.75	80.42
	% increase			1.95	8.40	20.12	35.15
24. IAW	average	42.8	40	41	45.5	59.45	81.98
	% increase				13.75	38.90	104.95
25. IMW	average	18	18.4	17.6	19	21.65	30.74
	% increase				3.26	20.27	67.07
26. WM	average	32.3	29	31	32	43.75	50
	% increase				10.34	35.45	72.41
27. TM	average	9.3	9.4	9	9.4	11.85	13.68
	% increase					27.42	45.53

TABLE III

MEASUREMENTS (IN MM.), PERCENTAGE INCREASE, AND PERCENTAGE RETARDATION OF ANCON SKULL

	Age	Newborn		2 months		6 months	Adult	
	Sex	♂	♀	♂	♀	♂	♂	♀
a. Length								
1. SKL	average	119.78	114.04	150.50	155.50	198.75	242.40	225.04
	% increase			25.64	36.35	65.39	102.37	97.33
	% retardation	—0.68	—3.68	+14.72	+5.07	—0.43		—3.29
2. BSK	average	103.30	98.58	125.80	137.00	173.25	214.57	197.40
	% increase			21.78	38.97	67.72	107.72	100.24
	% retardation	—1.43	—3.35	+9.20	+4.58	—2.26		—5.85
3. PL	average	58.09	55.94	73.70	76.70	99.03	129.67	121.25
	% increase			26.87	37.11	70.48	123.22	116.75
	% retardation	+2.27	+0.25	+12.69	+2.95	—3.15		—5.68
b. Width								
4. PmW	average	12.93	12.58	17.40	17.20	20.05	34.67	26.20
	% increase			34.57	36.72	55.07	68.13	102.63
	% retardation	—1.29	—10.14	+37.00	+39.83	—4.52		—6.49
5. IFT	average	42.11	41.15	49.00	51.6	61.93	84.2	75.36
	% increase			16.36	25.39	47.07	99.95	83.13
	% retardation	—5.58	—6.05	+3.16	+4.24	—3.23		—2.69
6. PtOW	average	67.87	65.88	91.30	89.40	106.95	126.90	120.39
	% increase			34.52	35.70	57.57	86.97	82.74
	% retardation	—2.06	—6.29	+12.44	+1.02	—1.60		—4.59
7. ZW	average	60.22	59.18	74.50	76.70	90.25	104.47	103.23
	% increase			23.71	29.60	49.86	74.06	74.43
	% retardation	—1.92	—4.55	+9.56	6.53	—5.74		—7.17
8. CW	average	51.93	50.99	64.60	61.60	68.75	77.30	65.98
	% increase			24.39	20.81	32.39	48.85	29.40
	% retardation	—2.93	—4.87	12.54	0.65	2.48		—0.06
c. Height								
9. CH	average	47.22	45.95	54.7	54.8	62.25	73.77	65.74
	% increase			15.84	19.26	31.83	56.23	43.07
	% retardation	+0.25	—0.11	+13.96	+7.03	—6.74		—4.50

TABLE III (*continued*)

	Age	Newborn		2 months		6 months	Adult	
	Sex	♂	♀	♂	♀	♂	♂	♀
10. SKH	average	55.24	54.8	70	71.1	90.73	114.27	100.09
	% increase			26.72	29.74	64.25	106.86	82.65
	% retardation	—1.09	+12.53	+20.69	+11.97	—0.73		0.01
11. FH	average	30.61	29.63	45.5	44.5	52.55	74.67	67.51
	% increase			48.64	50.24	71.68	143.94	127.84
	% retardation	+3.06	+3.79	+26.38	+12.66	8.61		—1.24
d. Occipital Region								
12. ICI	average	4.71	3.44	8	1.6	5.38	5.23	6.74
	% increase			96.5		14.23	11.04	95.93
	% retardation	—18.93	—52.88	+48.14	—98.00	—42.22		—17.81
13. DCW	average	13.51	4.85	17	5.35	17.98	15.07	20.31
	% increase			25.83	10.31	33.09	11.55	318.76
	% retardation	—22.85	—73.64	+6.25	—73.90	11.21		—4.15
14. SOFM	average	25.43	24.5	32.5	30.3	42.88	43.8	39.3
	% increase			27.80	23.67	68.62	72.24	60.41
	% retardation	—2.57	—7.55	+8.33	—2.26	—8.18		—3.77
15. SLFM	average	3.14	3.65	4.3	3.8	10.18	12.6	
	% increase			36.94	4.11	224.20	245.21	
	% retardation	—10.29	—40.00	—28.33	—33.33	26.76		
16. OcSp	average	15.46	15.45	18.8	21.05	20.3	28.9	24.18
	% increase			21.60	36.25	31.31	86.93	56.50
	% retardation	—7.98	—1.59	—7.43	—10.67	—22.52		—6.71
17. IPM	average	42.25	38.3	54	43.7	60.4	82.47	69.69
	% increase			27.81	14.10	42.96	95.19	81.53
	% retardation	—4.52	—5.19	+16.13	—17.55	—3.59		+1.53
18. BOW	average	15.39	14.43	20.5	16	23.2	29	25.61
	% increase			33.20	10.88	50.75	88.43	77.47
	% retardation	+3.29	—2.5	+24.24	—17.09	—4.53		—4.79

TABLE III (*continued*)

	Age	Newborn		2 months		6 months	Adult	
	Sex	♂	♀	♂	♀	♂	♂	♀
e. Mandible								
19. TL	average	85.94	83.63	107	111.1	142.5	177.6	178.45
	% increase			24.51	32.85	65.81	106.66	113.38
	% retardation	—3.65	—1.61	+1.90	—1.68	—5.35		—3.34
20. CSL	average	92.83	89.28	119.8	121.5	160.75	195.85	191.61
	% increase			29.05	36.09	73.17	110.98	114.61
	% retardation	+1.45	—5.52	+8.32	+1.67	—2.72		—2.83
21. H	average	38.16	38.66	56	61.8	86.55	108.75	108.46
	% increase			46.75	59.86	126.81	184.98	180.45
	% retardation	—1.39	—7.73	+1.08	+4.75	—4.79		—0.53
22. ICW	average	50.19	49.04	60.8	58.75	71	91.4	79.95
	% increase			21.14	19.80	41.46	82.11	63.03
	% retardation	—7.06	—9.19	+16.92	+1.29	+4.03		—1.13
23. IorW	average	52.47	51.09	60	59.7	68.38	93.5	77.81
	% increase			14.35	16.85	30.32	78.19	52.29
	% retardation	—6.97	—14.14	+4.34	—7.44	+0.93		—3.25
24. IAW	average	36.36	32.49	49	51.2	70.03	86.5	76.54
	% increase			34.76	57.59	92.60	137.89	135.58
	% retardation	—15.05	—18.77	+19.51	+12.53	+17.79		—6.64
25. IMW	average	17.52	16.8	18	17.35	21.78	31.25	28.09
	% increase			2.74	3.27	24.32	78.37	67.20
	% retardation	—2.67	—8.69	+2.27	—8.68	+0.6		—8.62
26. WM	average	29.83	28.94	35.7	36.4	40.38	49.8	47.34
	% increase			19.67	25.78	35.37	66.95	63.58
	% retardation	—7.65	—0.21	+15.16	13.75	—7.70		—5.32
27. TM	average	8.72	8.39	9.2	9.25	11.4	13.55	12.71
	% increase			5.50	10.25	30.73	55.39	51.49
	% retardation	—6.24	10.75	+2.2	—1.59	—3.79		—7.09

III. *Occipital region.* Measurements of the distance between the lateral occipital condyles show a greater fluctuation than any other part of the skeleton of Ancon sheep. The ventral distance of the normal animal also shows irregularity but to a much lesser extent. The percentage increase in the dorsal and ventral sides of the normal sheep, as one would expect, shows little alteration after birth. In the Ancon, due to the presence of more extremely abnormal cases among the newborn and intermediate stages, as compared with the adult, the relative increase in the adult is much higher than normal. The ventral distance between the lateral condyles in the adult Ancon female shows a retardation of 18%, which though more normal than in earlier stages, but is still the greatest retardation found in the adult skull.

B. Vertebrae

The actual sizes of all except the caudal vertebrae are given in Tables IV and V. Their percentages of increase between birth and maturity as well as the percentages of retardation (in parentheses) for adult females of both breeds are as follows:

	Height		Width		Length		Thickness	
	normal	Ancon	normal	Ancon	normal	Ancon	normal	Ancon
Cervical vertebrae	115.9	117.6 (2.1)	94.4	101.1 (1.5)	136.1	139.7 (1.1)	70.3	68.1 (2.9)
Thoracic vertebrae	150.5	123.0 (13.0)	78.4	79.9 (9.6)	124.7	128.6 (0.5)	80.8	57.9 (13.5)
Lumbar vertebrae	142.5	115.4 (15.4)	186.2	138.7 (2.5)	131.8	150.4 (0.2)	121.9	49.2 (32.4)
sacrum	119.1	150.0 (2.2)	157.2	158.0 (9.9)	121.9	131.0 (19.8)	78.8	58.0 (7.2)

From the columns for normal females in the above table it can be seen that each group of vertebrae has a maximum increase in a dimension that is characteristic for that type, viz., cervical vertebrae in length, thoracic in height, and lumbar in width and in thickness of centrum. The sacrum is complicated by sexual dimorphism; in the males the maximum relative increase is in height (Ancon male: height 160%, width 132.8%, length 27.5%, thickness of centrum 109%), in the females in width. The length of the sacrum fluctuates with the number of sacral vertebrae. In general, length of the vertebrae is only slightly retarded, while height, width and thickness of the centrum are much reduced, particularly in the thoracic and lumbar vertebrae.

TABLE IV

MEASUREMENT OF NORMAL VERTEBRAE (IN MM.)

Age		Newborn		2 months		6 mos.	Adult
Sex		♂	♀	♂	♀	♂	♀
Cervical Vertebrae	Height	28.3	24.6	28.8	30.3	53.5	53.1
	Width	31.9	28.5	30.1	31.9	51.2	55.4
	Length	17.5	15.5	18.5	17.3	30.3	36.6
	Thickness	12.8	11.8	12.4	13.4	17.4	20.1
Thoracic Vertebrae	Height	40.33	34.29		40.65		85.9
	Width	26.89	25.41		28.27		45.33
	Length	12.33	11.57		13.52		26
	Thickness	10.45	9.56		11.25		17.28
Lumbar Vertebrae	Height	28.33	25.2	27.1	29.3		61.1
	Width	48.4	40.19	43.5	45.5		115.03
	Length	15.33	15.2	17.2	18.8		35.24
	Thickness	10.93	9.6	10.5	11.5		21.3
Sacrum	Height	20.4	21	18.4	19	48	46
	Width	36.5	36	37.8	43.3	62	92.6
	Length	45	43	59.4	52.3	78.4	95.4
	Thickness	98	85	9	10.1	13.6	15.2

TABLE V

MEASUREMENT (IN MM.) OF ANCON VERTEBRAE

Age		Newborn		2 mos.	6 mos.	Adult	
Sex		♂	♀	♀	♂	♂	♀
Cervical Vertebrae	Height	25.18	23.9	36.6			52
	Width	27.65	26.3	38.5			52.9
	Length	15.41	15.1	28.3			36.2
	Thickness	11.9	11.6	17.5			19.5
Thoracic Vertebrae	Height	35.78	33.5	54.59			74.72
	Width	24.24	23.82	36.59			40.94
	Length	11.29	11.31	18.03			25.85
	Thickness	9.58	9.47	14.55			14.95
Lumbar Vertebrae	Height	24.72	24	37.7			51.7
	Width	38	36.4	66.1			86.9
	Length	14.8	14.1	36.4			35.3
	Thickness	9.97	9.65	13.3			14.4
Sacrum	Height	19.55	18	27	49.6	51	45
	Width	33.8	32.3	56.6	61	78.7	83.4
	Length	41.3	33	76	76.8	73.3	76.5
	Thickness	8.9	8.9	11.3	13.3	18.6	14.1

C. Ribs and thoracic cavity

There is no gross morphological abnormality characteristic of Ancon ribs. Measurements of the ribs and those of the thoracic cavity are given in Table VI. The percentages of increase and retardation of adult females are as follows:

		Percentage increase normal	Percentage increase Ancon	Percentage retardation Ancon
Rib	length	165.9	158.1	10
	width	156.9	150.8	15.5
Thoracic cavity	dorso-ventral diameter	122.6	128.1	13.5
	transverse diameter	192.8	188.2	1.7

Of all the individual bones in the skeleton of both breeds the ribs have the greatest percentage increase. However, the ribs of the Ancon are retarded, width relatively more than length.

The thoracic cavity of Ancon sheep is reduced in depth and nearly normal in width. The width/depth indices (Table VI) show the differential growth rate of depth and width in different ages. In the normal sheep the depth gradually decreases as the animal grows older. In Ancon sheep the same situation is found but it is more exaggerated. The difference between the two kinds of sheep, as shown by these indices, is already present in newborn lambs.

D. Extremities

Actual size as well as indices of the leg bones show a very narrow range of variation in the normal and a much wider range in the Ancon sheep. Absolute length of individual leg bones of Ancon varies from normal to nearly half or even less of the normal size, depending on which bone or segment is taken. Superficially the differences, in general, seem small in the newborn, but after two months of active growth, the symptoms of dwarfism become more and more evident. Actually, the differences, when expressed in percentages, are often as great or even greater in the newborn than in the older animals.

I. Forelimb

(Tables VII, IX, X, XI, XII, XIII, XIV)

1. *Scapula.* Tables VII, XIII show that normal and Ancon scapulae during early stages have very similar percentages of increase in

TABLE VI

MEASUREMENTS OF RIBS AND THORACIC CAVITY IN NORMAL AND ANCON SHEEP (IN MM.)

Age		Newborn		2 mos.		4 mos.	6 mos.	Adult	
Sex		♂	♀	♂	♀	♂	♂	♂	♀
Normal									
Ribs	length	79.16	70.02		89.5				186.15
	width	7.84	7.19		8.2				18.47
Thoracic cavity	dorso-ventral diameter	78	81	104	106				180.3
	transverse diameter	56.2	54.2	79	83				158.7
	$\frac{\text{D.V.D.}}{\text{T.D.}}$ index	1.373	1.494	1.316	1.277				1.136
Ancon									
Ribs	length	66.77	64.94						167.6
	width	6.27	6.22						15.6
Thoracic cavity	dorso-ventral diameter	75.5	68.4	89	121	138		183	156
	transverse diameter	57.3	52.4	78.2	88.7	111.5		171	151
	$\frac{\text{D.V.D.}}{\text{T.D.}}$ index	1.318	1.305	1.138	1.364	1.238		1.070	1.033

TABLE VII

MEASUREMENT (IN MM.), PERCENTAGE INCREASE AND PERCENTAGE RETARDATION OF BONY SCAPULAR

Age	Newborn		2 months		4 months	6 months	Adult	
Sex	♂	♀	♂	♀	♂	♂	♂	♀
Normal, length								
range	64-74.8				127.5-132.5			143.8-164.5
average	68.1	69.7	90.7	97.5	130	126.5	(158.5)	154.6
percentage increase			33.2	39.9	90.9	85.7	(132.7)	121.9
Normal, width								
range	42.6-50.9				97.5-100.9			105-119.5
average	46.3	47.5	58.5	64.5	99.2	88.5	(115.7)	113.3
percentage increase			26.2	35.8	114.0	90.9	(149.6)	137.5
Ancon, length								
range	53.55-70.0	55.5-72.5		82.6-102.2	88.5-121.5	109.7-130.3	135.5-158.5	133-150.2
average	63.36	60.98	92.9	92.4	114.4	119.2	146.6	143.0
percentage increase			46.6	51.5	80.5	88.2	131.4	134.5
percentage retardation	—7.0	—12.5	+2.4	—5.3	—12.0	—5.7		—7.5
Ancon, width								
range	38-46.9	37.8-49		61.5-74.9	65.5-90.8	84.5-91.4	101.3-116.9	94.4-117
average	43.4	41.9	63.2	68.2	85.3	88.3	110.2	107.9
percentage increase			45.4	62.6	96.5	103.4	153.6̊	157.3
percentage retardation	—6.3	—11.7	+7.9	+5.7	—13.97	—0.2		—4.7

TABLE VIII

MEASUREMENT (IN MM.), PERCENTAGE INCREASE, AND PERCENTAGE RETARDATION OF PELVIS

	Newborn		2 months		6 months	Adult	
	♂	♀	♂	♀	♂	♂	♀
Normal							
Length	93.9	90.0	102.5	107.5	163.0		220.2
			9.2	19.4	73.6		144.4
Width	27.9	27.8	31.8	34.0	42.0		68.1
			14.0	22.3	50.5		144.8
Tuber coxae	56.9	57.0	61.0	66.5	123.0		176.8
Distance			7.2	16.7	116.2		210.1
Conjugate	44.3	43.3	48.0	59	70.0		104.6
Diameter			8.5	36.3	58.2		141.6
Transverse	30.5	31.0	39.0	42.0	62.0		95.8
Diameter			27.9	35.5	100.0		209.0
Dorsal ischial	26.1	30.0	30.0	35.0	46.0		80.5
Proc. distance			14.9	16.7	76.2		168.3
Symphysial	28.3	29.0	31.4	30	54		51.5
Length			11.2	3.4	91.2		77.6
Ancon							
	88.4	84.8		141.5*		200.0	193.3
Length				66.86		126.4	127.9
	—5.9	—5.8					—12.3
	27.4	26.8		46.0		58.4	64.6
Width				71.9		113.99	141.53
	—1.83	3.8					—5.1
Tuber coxae	56.8	54.9		94.0		170	169.4
Distance				70.9		199.4	208.07
	—0.2	—3.5					—4.2
Conjugate	41.7	38.5		60.0		85.5	100.0
Diameter				55.8		103.9	159.7
	—5.8	—11.1					—4.4
Transversal	32	29.7		55.0		89	99.3
Diameter				85.37		178.1	234.7
	+4.9	—4.3					+3.7
Dorsal ischial	26.9	26.0		44.0		68.0	87.0
Proc. distance				69.4		152.5	234.9
	+3.2	—13.4					+8.1
Symphysial	27.2	26.1		47		63	44.8
Length				80.4		131.6	71.8
	—3.7	—10.2					—13.11

*This pelvis is 2½-month.

width and length but that of width exceeds that of length with age. Although the Ancon scapula is actually shorter and narrower than normal, it is relatively wider.

Based upon the data in Tables IX, X for length, Tables XI, XII for diameter and Tables XIII, XIV for diameter/length indices the following observations on the leg bones can be made:

TABLE IX

AVERAGE LENGTH (IN MM.), PERCENTAGE INCREASE, AND PERCENTAGE RETARDATION IN LONG BONES OF FORELEG

	Newborn		2 months		4 months	6 months	Adult	
	♂	♀	♂	♀	♂	♂	♂	♀
Normal humerus								
range	73.90.3				133.3-134.			147.6-157
average	79.7	80.4	97.5	107.65	133.68	127.9	(158.8)	148.5
% increase			22.4	33.9	67.8	60.6	(99.4)	84.6
Ancon humerus								
range	65.5-82.4	69.9-80.4	100-101.5	96.3-115.3	100-127.8	121.5-134	137.5-158.1	132.7-146.2
average	75.9	73.9	100.8	105.8	121.2	126.3	148.6	138.9
% increase			32.7	43.2	59.7	66.4	95.8	88.2
% retardation	—4.7	—8.2	+3.3	—1.7	—9.3	—1.3		—6.4
Normal Radioulna								
range	95.3-115.3				170.8-173.2			187.5-203
average	103.5	104.5	123.5	134.7	171.9	162.5	(228.7)	195.9
% increase			19.3	28.9	66.2	57.1	(120.9)	87.5
Ancon Radioulna								
range	78.1-103.2	79.3-92.5	111.8-121.5	97.9-115	100.3-158.8	115.8-164.5	120-189.9	113-169
average	89.3	86.8	116.6	106.5	138.8	141.2	163.4	140.0
% increase			30.7	22.7	55.5	58.2	83.1	61.3
% retardation	—20.2	—16.9	—5.5	—20.9	—19.3	—13.1		—28.53
Normal Metacarpus								
range	69.2-86.1				110.8-115			115.6-124.4
average	77.5	76.2	90.2	93.8	112.9	106.3	(142.5)	120.4
% increase			16.4	23.1	45.7	37.2	(83.9)	58.1
Ancon Metacarpus								
range	54.9-72.3	57.3-64.3	72.3-79.6	70.4-70.6	63.2-97.8	69.2-104.9	67.1-112.5	64.6-108.8
average	63.6	60.5	75.9	70.5	82.3	87.8	94.8	80.1
% increase			19.3	16.6	29.3	37.9	48.9	32.4
% retardation	—17.9	—20.6	—15.9	—24.8	—27.1	—17.5		—33.5

TABLE X

AVERAGE LENGTH (IN MM.), PERCENTAGE INCREASE, AND PERCENTAGE RETARDATION IN PHALANGES OF FORELEG
(M = MEDIAL; L = LATERAL)

	Newborn				2 months				4 months		6 months		Adult			
	♂		♀		♂		♀		♂		♂		♂		♀	
	M	L	M	L	M	L	M	L	M	L	M	L	M	L	M	L
Normal Phalanx I																
range	20–24.5	20–24.1	21.3–21.9	21.3–21.8	27.7–28	27.7–28	30.5–31	30	37.5–38	36.9–39	36.9–37.4	36.6–37			35.5–39	35.2–39
average	21.8	21.6	21.6	21.6	28	27.9	30.8	30	38	38	37.2	36.8			37.5	37.3
aver. M+L	21.7		21.6		27.9		30.4		37.9		36.9		(43.4)		37.4	
% increase					28.5		40.8		74.8		70.2		(99.6)		73.4	
Ancon Phalanx I																
range	19–23.2	18.8–22.5	18.8–21.4	18.5–23	24–28	24–27	23.4–25.6	25.6–28.3	26.4–39.7	25.2–38.4	27.–39	25.5–39	28.4–42.2	30–40.8	26.7–37	23.5–39
average	20.9	20.7	19.5	19.9	25.5	25.1	24.6	26.9	32.3	34.3	30.2	33.2	34.3	35.7	29.6	30.7
aver. M+L	20.8		19.7		25.3		25.8		32.7		31.7		34.9		30.2	
% increase					21.81		30.9		57.5		52.8		68.4		53.3	
% retardation	—4.4		—8.8		—9.4		—15.1		—13.9		—14.2				—19.3	
Normal Phalanx II																
range	14.4–17.8	14–17.9	16.2–16.5	16.2–16.4	20.2–20.5	19.1–19.8	20.8–21.8	20.8–21.5	25.5–26.6	25.4–26.3					24–26.3	23.5–27.6
average	16.2	15.9	16.3	16.3	20.4	19.5	21.3	21.2	25.9	26.1	25.3	25.4			25.2	25.3
aver. M+L	16.0		16.3		19.9		21.2		26.0		25.4		(26.76)		25.2	
% increase					24.1		30.0		62.2		58.0		(66.8)		54.6	
Ancon Phalanx II																
range	14–16.7	13.4–16.5	13.2–16	13.3–15.8	18–20	18–19.8	17.5–19.7	17.2–19.8	19.5–26	20.8–27	19.2–24	19.4–25	20–23	19.6–25.3	17.5–23.2	17.3–26.8
average	15.4	15.2	14.5	14.1	19.1	18.6	18.7	18.5	22.7	25.2	20.8	22.4	21.1	22.5	19.7	20.5
aver. M+L	15.3		14.3		18.9		18.6		23.9		21.6		21.8		20.1	
% increase					22.2		29.7		56.2		41		41.9		31.1	
% retardation	—4.5		—12.4		—5.2		—12.6		—8		—14.8				—20.4	

TABLE XI

AVERAGE DIAMETER (IN MM.), PERCENTAGE INCREASE AND PERCENTAGE RETARDATION IN LONG BONES OF FORELEG

	Newborn		2 months		4 months	6 months	Adult	
	♂	♀	♂	♀	♂	♂	♂	♀
Normal humerus								
range	8.9-10.9				17.4-18.4			19-21
average	9.8	9.0	10.9	11.3	17.9	16.4	(21.1)	20
% increase			11.57	25.6	82.7	67.9	(116.3)	104.5
Ancon humerus								
range	8.6-11.1	8.3-10	11.4-11.8	10.8-13.3	11.5-17.4	14.8-17.2	17.4-20	16.8-19.9
average	9.8	9.1	11.6	12	15.8	15.7	19.1	18.1
% increase			18.9	32.2	62.2	60.5	95.7	98.9
% retardation	—1.03	+0.9	+0.26	+6.19	—13.2	—4.5		—9.2
Normal radioulna								
range	10.5-12.8				18.5-18.6			20.3-21.6
average	11.5	10.9	11.4	12.5	18.5	17.9	(21.3)	20.9
% increase			1.1	14.75	61.4	55.5	(85.5)	92.4
Ancon radioulna								
range	11.3-14.4	11.3-12.3	14.2-16.4	16-18.5	17.4-23.5	16.6-19.9	21-28.2	18-26.5
average	12.9	11.8	15.3	17.3	20.5	18.5	20.0	22.6
% increase			18.4	46.6	58.4	43.3	78.3	92.0
% retardation	+12.5	+8.5	+134.8	+38.6	+10.5	+3.7		+8.2
Normal metacarpus								
range	9.9-11.6				16.2-17.3			16-17.6
average	10.5	10.2	11.5	12.4	16.8	15.9	(18.7)	16.6
% increase			9.3	22.2	59.2	50.7	(56.2)	63.4
Ancon metacarpus								
range	10.3-12.4	10.2-11.4	12.9-13.2	12.2-14.1	13.1-17.5	15.3-16.9	17.6-21.7	15.5-18.6
average	11.5	10.7	13.1	13.1	16	16	19.6	17.4
% increase			13.5	13.7	38.7	39.2	70.4	51.5
% retardation	+9.3	+5.7	+13.48	+10.8	+4.78	+1.0		+5.1

TABLE XII

AVERAGE DIAMETER (IN MM.), PERCENTAGE INCREASE AND PERCENTAGE RETARDATION IN PHALANGES OF FORELEG
(M = MEDIAL; L = LATERAL)

	Newborn				2 months				4 mos.		6 mos.		Adult			
	♂		♀		♂		♀		♂		♂		♂		♀	
	M	L	M	L	M	L	M	L	M	L	M	L	M	L	M	L
Normal																
Phalanx I					8.8–	9.4–	9.8–	9.6–	12–	12–	12.2–	11.8–			12–	12–
range	7.5–	7–	8.4–	8.1	10.3	9.6	10.3	9.8	13.3	13	13.1	13.3			13.2	13
	9.9	9.7	8.5		9.6	9.5	10.1	9.7	12.7	12.5	12.7	12.6			12.5	12.4
average	8.7	8.5	8.5	8.1	9.5		9.9		12.6		12.6		(13.2)		12.4	
avr. M+L	8.6		8.3		10.9		19.3		46.2		46.7		(54.0)		50.2	
% increase																
Ancon																
Phalanx I																
range	8–	7.8–	7.3–	7–	10.4–	10.5–	10.5–	9.4–	10.3–	11–	11.7–	11.5–	12.5–	11.4–	12.8–	12.5–
	10.2	9.7	9.3	8.4	12.2	11.4	12.2	12.4	13.7	12.5	15	14.3	20	16.4	15	16.2
average	9.2	8.8	8.2	7.8	11.4	11.1	11.4	10.8	12.1	11.7	13.5	12.9	15.9	13.7	14	14.1
avr. M+L	9.0		8.0		11.3		11.0		12		13.2		14.8		14.0	
% increase					24.8		37.1		32.7		46.3		64.4		75.0	
% retardation	+5.0		—3.3		+18.2		+11.1		—4.7		+4.8				+12.7	
Normal																
Phalanx II																
range	6.5–	6.2–	6.4–	6.4	8	7.5–	8.8–	8.6–	10.8–	10.8–					10.5–	10.4–
	8.8	8.5	6.6			7.8	9.4	9	11.5	11					11.3	11.4
average	7.7	7.3	6.5	6.4	8	7.7	9.1	8.8	11	11	11.6	11			11	10.8
avr. M+L	7.5		6.5		7.8		9		11		11.3		(11.7)		10.9	
% increase					4.4		38.8		46.0		50.7		(55.5)		69.2	
Ancon																
Phalanx II																
range	7–	6.6–	6.2–	6.4–	9.5–	9.2–	9.5–	9–	10–	9.6–	11.9–	11–	12–	10–	10.7–	11–
	8.7	8.7	8.2	8	10.2	10	11.4	11.2	13.7	12.4	13	13	15	14.7	13.5	13.2
average	7.9	7.7	7.1	7.0	9.8	9.7	10.4	10.2	11.6	10.8	12.5	11.8	13.2	12.4	12.2	11.8
avr. M+L	7.8		7.1		9.7		10.3		11.2		12.1		12.8		12	
% increase					24.6		45		43.4		71.7		64.1		69.7	
% retardation	+4.3		+9.6		+24.4		+14.5		+2.4		+7.43				+10.0	

TABLE XIII

TABLE XIII

INDICES OF DIAMETER/LENGTH OF FORELEG

		Scapular		Humerus		Radioulna		Metacarpus	
		range	avr.	range	avr.	range	avr.	range	avr.
Normal	♂	.6646–.6956	.6802	.1202–.1263	.1228	.1052–.1147	.1102	.1322–.1421	.1328
New-born	♀		.6819		.1119		.1038		.1333
2 mo.	♂		.6451		.1118		.0919		.1275
	♀		.6616		.1049		.0925		.1323
4 mo.	♂	.7612–.7647	.7629	.1294–.1377	.1336	.1075–.1081	.1078	.1409–.1563	.1486
6 mo.	♂		.6928		.1282		.1099		.1491
Adult	♀	.6718–.8313	.7354	.1267–.1373	.1328	.1035–.1105	.1067	.1335–.1416	.1377
Ancon	♂	.6398–.7367	.6868	.1214–.1459	.1288	.1164–.2093	.1481	.1587–.2002	.1815
New-born	♀	.6645–.7397	.6876	.1138–.1326	.1236	.1277–.1552	.1359	.1642–.1907	.1776
2 mo.	♂		.6798	.1124–.1180	.1152	.1169–.1468	.1319	.1659–.1785	.1722
	♀	.7333–.7446	.7389	.1117–.1149	.1152	.1609–.1665	.1637	.1729–.1997	.1863
4 mo.	♂	.7015–.7854	.6402	.1145–.1392	.1302	.1209–.1825	.1501	.1627–.2465	.1987
6 mo.	♂	.7015–.7707	.7429	.1213–.1284	.1299	.1086–.1658	.1346	.1589–.2372	.1881
Adult	♂	.7374–.7689	.7517	.1183–.1339	.1286	.1114–.2134	.1530	.1560–.3167	.2200
	♀	.7302–.8523	.7635	.1219–.1409	.1299	.1065–.2214	.1651	.1476–.2764	.2249

TABLE XIV

INDICES OF DIAMETER/LENGTH IN PHALANGES OF FORELEG

		side	1st phalanx range	1st phalanx aver.	1st phalanx average of Med.+Lat.	2nd phalanx range	2nd phalanx aver.	2nd phalanx average of Med.+Lat.
Normal	♂	M	.3606–.4286	.3986		.4333–.5029	.4736	
					.3921			.4665
		L	.3448–.4135	.3855		.4428–.4749	.4593	
Newborn		M	.3836–.3991	.3914		.3951–.4000	.3976	
	♀				.3873			.3952
		L	.3716–.3803	.3759		.3902–.3951	.3927	
		M	.3166–.3653	.3409		.3902–.3960	.3931	
2 mo.	♂				.3411			.3934
		L	.3357–.3466	.3412		.3788–.4084	.3936	
		M	.3213–.3323	.3268		.4231–.4312	.4272	
	♀				.3251			.4217
4 mo.		L	.3200–.3267	.3234		.4135–.4186	.4161	
	♂	M	.3209–.3333	.3379		.4235–.4323	.4296	
					.3356			.4246
		L	.3125–.3547	.3332		.4183–.4252	.4196	
6 mo.		M	.3306–.3503	.3405			.4585	
	♂				.3409			.4458
		L	.3189–.3634	.3412			.4331	
Adult		M	.3205–.3507	.3329		.4154–.4750	.4371	
	♀				.3331			.4330
		L	.3158–.3494	.3332		.3841–.4609	.4289	
Ancon								
	♂	M	.3220–.4904	.4357		.4724–.6000	.5168	
					.4324			.5117
		L	.3556–.4778	.4291		.4416.–5800	.5065	
Newborn	♀	M	.3850–.4650	.4195		.4583–.5467	.4916	
					.4066			.4945
		L	.3565–.4433	.3936		.4610–.5329	.4974	
		M	.3714–.4939	.4500		.5000–.5278	.5129	
2 mo.	♂				.4385			.5174
		L	.3392–.4750	.4270		.4647–.5625	.5220	
	♀	M	.4430–.4766	.4646		.5278–.5787	.5519	
					.4587			.5498
4 mo.		L	.3322–.4697	.4528		.5143–.5657	.5477	
	♂	M	.2953–.5112	.3862		.3846–.6473	.5245	
					.3663			.4783
		L	.2933–.4365	.3463		.3769–.5962	.4321	
6 mo.	♂	M	.3120–.5475	.4440		.5292–.6404	.6039	
					.4215			5668
		L	.3194–.5490	.3989		.4400–.5876	.5296	
	♂	M	.2976–.6667	.4837		.5565–.7143	.6277	
					.3912			.5926
Adult		L	.2822–.5467	.3912		.4120–.6682	.5575	
	♀	M	.3811–.5556	.4704		.4914–.7429	.6216	
					.4723			.6036
		L	.3247–.6894	.4741		.4366–.7174	.5855	

TABLE XV

AVERAGE LENGTH (IN MM), PERCENTAGE INCREASE AND PERCENTAGE RETARDATION IN LONG BONES OF HINDLEG

	Newborn		2 months		4 months	6 months	Adult	
	♂	♀	♂	♀	♂	♂	♂	♀
Normal Femur								
range	81.9-99.6				158.3-164.3			175.4-189.3
average	88.5	89.8	113.6	121.9	161.3	153.8	(198.7)	182.6
% increase			28.4	35.8	82.3	73.9	(124.6)	103.5
Ancon Femur								
range	71.8-92.1	77.7-85.8	103.9-117.2	97.6-133.5	100.3-152	138.7-157.8	136.9-187.9	140.2-182
average	83.5	80.4	110.5	115.6	157.6	143.9	173.2	158.7
% increase			32.5	43.7	64.93	72.4	107.5	97.4
% retardation	—5.7	—10.4	—2.7	—5.2	—14.7	—6.5		—13.1
Normal Tibia								
range	98.1-117.5				177-183.7			199.5-219
average	105.2	104.0	130.0	138.2	180.4	179.1	(218.1)	209.1
% increase			23.6	32.9	71.4	70.3	(107.3)	101.0
Ancon Tibia								
range	72.8-95.2	76.8-84.5	100.9-113.5	95.5-119	109.9-139.1	129.5-147.5	157.4-192.2	148.2-175
average	83.7	82.1	107.2	107.3	127.7	135.2	168.2	161.2
% increase			29.0	30.6	52.5	61.4	100.8	96.3
% retardation	—20.4	—21.0	—17.6	—22.4	—29.2	—24.5		—22.9
Normal Metatarsus								
range	70.3-87.9				111.2-115.7			116.8-124.8
average	78.7	78.2	90.9	94.3	113.4	109.1	(127.0)	120.5
% increase			15.4	20.6	44.1	39.7	(61.3)	54.1
Ancon Metatarsus								
range	53.6-70.5	56.2-61.4	64-72.2	64.8-67.5	60.4-73.6	65.7-75	63.7-75.8	63.7-77
average	61.0	58.7	68.1	66.1	68.7	69.9	72.4	68.6
% increase			11.6	12.6	12.5	14.5	18.6	16.9
% retardation	—22.5	—24.9	—25.1	—29.8	—39.5	—35.9		—43.0

2. *Humerus.* In comparison with the normal, both the percentage retardation and the diameter/length indices show more narrowing than shortening in the humeri of the Ancon. Judging by the indices, the normal bone tends to become wider as the animal grows older, but the Ancon humerus fails to show this change due to its relatively greater retardation in width. By studying the range of variation in length, one can see that even the shortest bone increases with age. This would indicate that each bone is able to grow, though slowly.

3. *Radius-ulna.* The range of actual length and of diameter/length indices show that, after the second month of postnatal life, some of the Ancon bones are normal in the increase of length, whereas others are arrested. The average percentage increase for length in the adult is about 2/3 of the normal. The percentage retardation is already high in the newborn (male 20%, female 17%) and slightly greater in the adult female (28.5%).

The diameter of the Ancon radius-ulna rapidly increases during the first two months, after which it slows down to the same rate as in normal; but actually the bone is wider in all stages. The percentage retardation in this case is the percentage of widening. The increase in diameter, as will be shown in a separate report, is chiefly due to flattening of the radius and the lateral position of the ulna.

4. *Metacarpus.* Judging from the range of variation in length, most of the metacarpi are of the extreme type and cease to grow in length prior to the second month; the remainder stop their increase after the 6th month; metacarpi of normal sheep, on the other hand, remain active beyond this stage. None of the Ancon metacarpi, therefore, reach normal size in any stage. The normal metacarpus shows less postnatal growth than any other segment of the foreleg. In the Ancon this lack of growth is even more exaggerated. While the increase in length is arrested, the diameter of the Ancon metacarpus keeps increasing slowly so that the diameter-length ratio increases with age. The normal metacarpus has only negligible amount of increase. This bone of the adult Ancon female is, on the average, 33.5% shorter and 5% wider than the normal.

During measurement it was noticed that in Ancons the sagittal notch at the distal end of the cannon bone was irregular and the medial and lateral condyles were not at the same level. The measurements were then changed to include distances: (1) from the ridge between the two

articular facets of the proximal end to the distal ridge of the articular surface of the medial condyle, and (2) to the corresponding point of the lateral condyle. The lateral length is then divided by the medial as shown in Table XVI. The indices thus derived show negligible differences in normal sheep (less than 0.5%), whereas in Ancons the lateral side is, on the average, shorter than the medial (3% in the newborn, 6% in the adult).

In the normal cases the maximal difference between the two sides is 1.1%. By assuming a 2% difference as the maximal normal range, and those more than 2% as abnormal, the following frequencies were found:

	Medial side over 2% longer	Normal range (<2%)	Lateral side over 2% longer	Total No. of bones
Newborn	24	14	0	38
2-month	7	1	0	8
4-month	3	9	0	12
6-month	1	7	2	10
Adult	9	18	0	27
Total No. of bones	44	49	2	95
Percentage of cases	46.3	51.6	2.1	

The above tabulation shows that there are many more cases with the medial side longer than with the lateral.

5. *Phalanx I.* The maximal range of length in each stage of the Ancon sheep is the same as in the normal. The upper limit of the minimal range is greater in each successive stage than in the previous stage indicating that the affected bones are only hypoplastic and that none of them is completely arrested in any stage. Both the normal and the Ancon phalanges increase only slightly after the fourth month.

The cessation of increase in diameter in normal sheep coincides with cessation of increase in length, but in Ancon the former continues after the completion of the latter. The result is that the phalanges of newborn Ancons are 4% shorter and 5% wider than the normal ones and those of the adult are 19% shorter and 12% wider. The width-length ratio normally decreases with advancing age, but the reverse is true in Ancons.

The averages of length, width and diameter/length ratio show the medial side of Ancon phalanges to be shorter and wider than the

lateral. The indices of medial phalangeal length divided by lateral phalangeal length show no significant difference between the averages of the two sides in the normal sheep but there is a definite difference in the Ancon which is especially clear in the female material.

If we treat the medial-lateral ratio of the phalanges with the same method used for the metacarpus, the following frequencies were found:

	Medial side over 10% longer	Normal range (<10%)	Lateral side over 10% longer	Total No. of cases
Newborn	0	35	2	37
2-month	0	6	2	8
4-month	0	7	3	10
6-month	0	6	3	9
Adult	3	15	9	27
Total no. of bones	3	69	19	91
Percentage of cases	3.3	75.8	20.9	

In 21% of the cases the lateral side is over 10% longer and only 3% belong to the group in which the medial side is the longer one. It is interesting to notice that this situation is opposite to the condition seen in the metacarpus.

6. *Phalanx II.* The growth of the second phalanx is very similar to that of the first. However, the completion of increase in length is earlier in both kinds of sheep than in phalanx I and the length retardation is greater. The media-lateral sides are again different in length and width. By using the same grouping method as used before the following results are found:

	Medial side over 10% longer	Normal range (<10%)	Lateral side over 10% longer	Total No. of bones
Newborn	0	35	0	35
2-month	1	7	0	8
4-month	0	5	3	8
6-month	1	3	3	7
Adult	1	17	6	24
Total No. of bones	3	67	12	82
Percentage of cases	3.7	81.7	14.6	

From the above tabulation we see that there are 14.6% of cases with the lateral side over 10% longer, whereas the greater length of the medial side was found in only 3.7%. Although the second phalanx

is more retarded in length, the bilateral difference is less severe than that observed in the first. Again the difference is opposite to that found in the metacarpus.

II. Hindlimb

(Tables VIII, XV, XVI, XVII, XVIII, XIX, XX)

1. *Pelvis.* (Table VIII)—The pelvis of normal as well as Ancon sheep is subject to sex dimorphism. Apart from this sex dimorphism, the length of the pelvis of Ancon sheep is more reduced (12%) than the width of each girdle (5%) and the inter-tuber-coxae distance (4%). The conjugate diameter is smaller (4%) but the transverse diameter (4%) and the distance between the dorsal ischial processes (8%) are actually wider than in the same sex of the normal. The symphyseal length in Ancon, like the total length, shows a high percentage of retardation (13%).

2. *Femur.* The ranges of length show that some of the femora of Ancon sheep are normal in length while others had a growth rate slower than normal; none of them had a complete arrest in any stage. A few extreme cases, as shown by the maximal range of width, are actually wider than normal, but most of them are narrower.

In normal sheep the percentages of increase in length and width of the femur between birth and maturity were the same (103.5%). In Ancons the relative increase in length is greater (97.4%) than that in width (92.6%).

3. *Tibia.* Judging by the range of the length measurements none of the Ancon tibiae reach the normal length; nor is there a complete arrest in any stage. In normal sheep the percentages of increase in length (101%) and width (104%) are nearly the same, but in Ancons that in width (71%) is less than that in length (96%). The Ancon tibia, more or less similar to its homologue, the radius, has a pronounced micromelic expression in the newborn lamb. The adult tibia is not as greatly reduced (23%) as the radius-ulna, but it is more reduced in all other stages.

4. *Metatarsus.* The range of variation in length shows that the growth in some of the metatarsal bones is normal in the first two months whereas the rest of them are, at best, very slow. On the average the growth of the metatarsus does not show the rapid increase during the first two months that is typical of the other bones; in fact, its

TABLE XVI

AVERAGE LENGTH (IN MM.), PERCENTAGE INCREASE AND PERCENTAGE RETARDATION IN PHALANGES OF HINDLEG (M — MEDIAL; L — LATERAL)

	Newborn				2 months				4 mos.		6 mos.		Adult			
	♂		♀		♂		♀		♂		♂		♂		♀	
	M	L	M	L	M	L	M	L	M	L	M	L	M	L	M	L
Normal Phalanx I																
range	20–23.8	19.8–23.9	21.2–21.6	21.7–22	27	26.7–27	28.7–29	29	37.4–38.6	37.8–36.3	36–36.3	36.5			35–40	34–40
average	21.6	21.5	21.9	22	27	26.9	28.9	29	38	38	36.2	36.5			37.2	37.1
avr. M—L	21.5		21.6		26.9		28.9		38		36.3		(40.5)		37.2	
% increase					25.1		33.8		76.5		68.8		(88.1)		71.8	
Ancon Phalanx I																
range	19–23	19–23.4	18.4–21.4	18.4–22	25–27	25.2–27.5	23–27	24–25.7	25–28.7	25.7–29.2	26.7–35.8	25.7–29.2	26–41.7	25.5–42.3	25–39	25–40
average	20.7	21	19.6	19.9	26	26.2	24.8	24.6	26.6	32.9	27.3	29.6	30.7	32.3	27.9	30.0
avr. M—L	20.9		19.8		26.1		24.7		29.7		28.5		31.5		28.9	
% increase					25		24.8		42.5		36.5		51.0		46.4	
% retardation	—3.0		—8.6		—3.2		—14.7		—21.7		—21.6				—22.1	
Normal Phalanx II																
range	14.7–17.4	14.2–17.7	16–16.2	16.4	19.2–19.5	19–19.4	21–21.4	21.4–22.3	25.5–26.5	26–26.8					23.5–25.7	24.7–26.7
average	16.1	16.1	16.1	16.4	19.4	19.2	21.2	21.9	26.1	26.5	26	25.8			24.5	25.4
avr. M—L	16.1		16.3		19.3		21.5		26.3		25.9		(26.2)		25	
% increase					20.2		32.5		63.7		57.9		(63.5)		53.7	
Ancon Phalanx II																
range	13.8–16.2	14.2–16.5	12–15.8	13.5–16.4	17.5–18.4	18–19	16.7–18.8	16.7–19.6	18–20	20–27	18.5–21	18.8–21.7	18.5–21	18–27.7	17–20.5	17–21.3
average	15.0	15.1	14.5	14.9	18	18.6	17.5	17.7	18.9	21.8	19.3	20.1	19.3	20.3	19	18.8
avr. M—L	15.1		14.7		18.3		17.6		20.1		19.7		19.8		18.9	
% increase					21.3		20.1		33.2		30.4		31.4		28.9	
% retardation	—6.0		—9.8		—5.1		—18.3		—23.5		—24.1				—24.4	

TABLE XVII

AVERAGE DIAMETER (IN MM.), PERCENTAGE INCREASE AND PERCENTAGE RETARDATION IN LONG BONES OF HINDLEG

	Newborn		2 months		4 months	6 months	Adult	
	♂	♀	♂	♀	♂	♂	♂	♀
Normal Femur								
range	9.8-11.7				16.9-17.5			19.4-20
average	10.7	9.75	11.6	12.2	17.2	17.2	(19.9)	19.8
% increase			8.9	25.1	61.0	61.0	(86.6)	103.5
Ancon Femur								
range	9.4-12.5	8.6-10.4	11.4-11.5	11.4-13.9	12-18.8	14.2-15.4	16.9-20.1	16.5-22.8
average	10.3	9.6	11.4	12.6	15.3	14.8	18.5	18.4
% increase			11.4	32.0	48.8	44.3	79.9	92.6
% retardation	—3.7	—1.8	—1.5	+3.5	—9.6	—13.7		—7.1
Normal Tibia								
range	9.7-11.4				16.2-16.6			17.7-20.8
average	10.3	9.2	10.9	11.3	16.4	15.0	(18.9)	18.7
% increase			6.3	23.5	59.8	46.3	(84.2)	103.8
Ancon Tibia								
range	10-12.7	9.3-11.2	11.8-12.3	11.6-13.7	11.3-15.9	14-15.7	16.5-19.6	16.4-18.5
average	10.8	10.1	12.0	12.6	14.5	14.8	17.6	17.4
% increase			11.0	24.7	33.6	36.5	62	71.3
% retardation	+5.8	+10.7	+0.7	+11.8	—11.6	—1.3		—7.0
Normal Metatarsus								
range	9-10.5				14			14-14.9
average	9.5	9	10.6	11.1	14	14	(15.9)	14.3
% increase			12.2	22.8	48.1	48.1	(68.9)	58.3
Ancon Metatarsus								
range	9-11.4	9-10.5	11.2-11.5	10.6-12.4	10.6-14.9	12.5-14.7	14.7-16.3	12-15.2
average	10.4	9.6	11.3	11.5	13.6	13.5	15.6	13.9
% increase			9.2	19.2	31.2	30.6	50.5	44.4
% retardation	+9.5	+7.0	+6.6	+3.89	—3.0	—3.4		—2.4

TABLE XVIII

AVERAGE DIAMETER (IN MM.), PERCENTAGE INCREASE AND PERCENTAGE RETARDATION IN PHALANGES OF HINDLEG (M — MEDIAL; L — LATERAL)

	Newborn ♂		Newborn ♀		2 months ♂		2 months ♀		4 mos. ♂		6 mos. ♂		Adult ♂		Adult ♀	
	M	L	M	L	M	L	M	L	M	L	M	L	M	L	M	L
Normal Phalanx I																
range	7–9.4	6.6–8.8	7.4–7.5	7.3–7.4	8.5–9	8.3–9	9.5–10.3	8.3–10	11.5	10.5–12.2	12–12.3	10.7–11			11–13	11–12.4
average	8.3	7.8	7.5	7.4	8.8	8.7	9.9	9.2	11.5	11.3	12.2	10.9			12	11.7
avr. M+L	8.1		7.4		8.7		9.5		11.4		11.5		(12.5)		11.8	
% increase					8.1		28.8		41.9		42.9		(54.8)		59.9	
Ancon Phalanx I																
range	7.5–10.5	7.3–9.7	7.3–8.8	6.8–8.6	9.6–10.5	10–10.3	9.4–11	9–10.6	9–14	10.3–12.4	11.6–14	10.3–14.3	12.3–18	11–15.2	12–14.2	11–14.5
average	9.3	8.4	7.9	7.7	10.1	10.1	10.1	9.7	12.3	11.4	12.7	12.1	13.9	13.2	13.3	12.4
avr. M+L	8.9		7.8		10.2		10.0		11.9		12.5		13.5		12.9	
% increase					14.5		28.8		34.8		40.8		53		65.1	
% retardation	+9.9		+5.3		+16.4		+5.3		+4.5		+8.4				+8.7	
Normal Phalanx II																
range	6.2–7.8	6.2–8	6	5.6–5.9	7.4–7.5	6.8–7	7.8–8.8	8–8.7	9.4–9.8	9.4–10					9.5–10.3	9–10
average	7.0	6.7	6	5.8	7.5	6.9	8.3	8.4	9.6	9.5	9.7	9.3			9.8	9.4
avr. M+L	6.9		5.9		7.2		8.3		9.5		9.5		(9.9)		9.6	
% increase					4.4		41.7		38.2		31.5		(44.9)		63.4	
Ancon Phalanx II																
range	6.3–8.9	6–9	6.2–7.6	5.8–7.6	8.6–9.3	8.4–9.5	8.6–10.7	9–9.6	9.5–12	9–12.3	10.6–12	10.4–11.3	11–12.8	9.7–13	10–12.4	9.6–12
average	7.5	7.1	6.9	6.7	8.9	8.8	9.5	9.2	10.9	10.5	11.1	10.6	11.8	11.2	11.1	11
avr. M+L	7.3		6.8		8.8		9.4		10.7		10.8		11.5		11.1	
% increase					21.3		37.9		46.8		48.7		57.5		62.7	
% retardation	+6.0		+15.7		+23.1		+12.6		+12.5		+14.1				+15.1	

TABLE XIX

INDICES OF DIAMETER/LENGTH IN LONG BONES OF HINDLEG

		Femur		Tibia		Metatarsus	
		range	average	range	average	range	average
Normal							
Newborn	♂	.1156–.1283	.1205	.0965–.0989	.0975	.1152–.1266	.1202
	♀		.1087		.0879		.1151
2 mo.	♂		.1022		.0838		.1167
	♀		.1001		.0818		.1173
4 mo.	♂	.1062–.1065	.1064	.0901–.0916	.0909	.1210–.1259	.1235
6 mo.	♂		.1114		.0838		.1283
Adult	♀	.1057–.1140	.1086	.0833–.0975	.0892	.1156–.1205	.1184
Ancon							
Newborn	♂	.1075–.1509	.1234	.1083–.1562	.1302	.1504–.1852	.1703
	♀	.1057–.1268	.1188	.1121–.1338	.1234	.1490–.1774	.1642
2 mo.	♂	.0973–.1104	.1039	.1040–.1215	.1128	.1547–.1789	.1668
	♀	.1042–.1164	.1103	.1152–.1209	.1181	.1629–.1837	.1740
4 mo.	♂	.0961–.1426	.1121	.1007–.1238	.1134	.1748–.2207	.1811
6 mo.	♀	.0976–.1066	.1023	.1065–.1143	.1096	.1889–.1994	.1934
Adult	♂	.0949–.1473	.1087	.0985–.1090	.1046	.2021–.2419	.2134
	♀	.0993–.1513	.1154	.0970–.1249	.1079	.1739–2279	.2034

TABLE XX

INDICES OF DIAMETER/LENGTH IN PHALANGES OF HINDLEG

		side	1st phalanx range	1st phalanx aver.	1st phalanx average of Med.+Lat.	2nd phalanx range	2nd phalanx aver.	2nd phalanx average of Med+Lat.
Normal	♂	M	.3145–.4126	.3832	.3741	.4218–.4483	.4360	.4350
Newborn		L	.3333–.3981	.3649		.4133–.4624	.4340	
	♀	M	.3472–.3491	.3482	.3423	.3704–.3750	.3725	.3616
		L		.3364		.3415–.3598	.3507	
2 mo.	♂	M	.3418–.3333	.3241	.3224	.3846–.3854	.3850	.3722
		L	.3109-.3333	.3206		.3579–.3608	.3594	
	♀	M	.3276–.3589	.3433	.3294	.3645–.4191	.3918	.3872
		L	.2682–3448	.3155		.3587–.4065	.3826	
4 mo.	♂	M	.2979–.3075	.3031	.3006	.3547–.3843	.3664	.3624
		L	.2749–.3228	.2981		.3358–.3774	.3583	
6 mo.	♂	M	.3306–.3417	.3362	.3168		.3731	.3668
		L	.2932–.3014	.2973			.3605	
Adult	♀	M	.3099–.3429	.3226	.3171	.3697–.4255	.3999	.3854
		L	.2902–.3296	.3115		.3557–.4000	.3709	
Ancon	♂	M	.3907–.5000	.4265	.4147	.4400–.5867	.4965	.4826
Newborn		L	.3216–.4686	.4028		.4189–.5867	.4687	
	♀	M	.3700–.4433	.4052	.3945	.4194–.5333	.4751	.4647
		L	.3500–.4300	.3838		.3946–.5000	.4543	
2 mo.	♂	M	.3556–.4200	.3919	.3888	.4889–.5054	.4964	.4834
		L	.3745–.3968	.3856		.4526–.5000	.4703	
	♀	M	.4074–.4115	.4092	.4067	.5059–.5692	.5460	.5338
4 mo.		L	.3750–.4250	.4042		.4898–.5389	.5215	
	♂	M	.3557–.5056	.4613	.4081	.5278–.6053	.5744	.5297
		L	.2956–.4429	.3549		.3600–.5750	.4850	
6 mo.	♀	M	.4349–.5263	.4650	.4423	.5429–.6316	.5775	.5513
		L	.2901–.5112	.4196		.4839–.5795	.5271	
Adult	♂	M	.2950–.6122	.4663	.4450	.5714–.6667	.6084	.5856
		L	.2106–.5630	.4237		.3502–.6631	.5626	
	♀	M	.3333–.5320	.4808	.4532	.4878 .6706	.5887	.5877
		L	.2750–.5179	.4256		.5052–.6765	.5866	

growth is to an exceptional degree slower than normal; after the second month there is only a negligible amount of increase. The percentage increase in length of the adult female (17%) is less than 1/3 of the normal (54%). The width of the Ancon metatarsus is similar to that of the tibia, that is, wider than normal (7%) in the newborn but narrower (2.4%) in the adult. The percentage retardation in length is high in the newborn (22.5%) and doubled in the adult (43%).

The metatarsus shows a medial-lateral difference. When the individual indices are grouped into 3 categories, the results are as follows:

	Medial side over 2% longer	Normal range (<2%)	Lateral side over 2% longer	Total No. of bones
Newborn	4	34	0	38
2-month	1	6	0	7
4-month	1	11	0	12
6-month	2	8	0	10
Adult	7	19	1	27
Total No. of bones	15	78	1	94
Percentage of cases	15.9	82.9	1.1	

The metatarsus resembles the metacarpus in that there are more cases with the lateral side shorter than the medial and rarely the reverse. Although the metatarsus is more retarded, its medio-lateral difference is not as great as in the latter.

5. *Phalanx I.* The most affected phalanges fail to increase in length after the second month, but all of them continue to grow in width. The phalanx I in newborn Ancons is, on the average, 3% shorter than normal, but in adults this difference is much more exaggerated (22%). The diameter of the bone in the newborn, on the contrary, is greater than in the normal; and this condition is slightly intensified in the adult.

As in the phalanges of the foreleg, the phalanges of the hindleg show medio-lateral differences. The frequencies of these differences follow:

	Medial side over 10% longer	Normal range (<10%)	Lateral side over 10% longer	Total No. of bones
Newborn	0	36	1	37
2-month	0	7	0	7
4-month	0	4	6	10
6-month	0	7	2	9
Adult	2	19	6	27
Total No. of bones	2	73	15	90
Percentage of cases	2.2	81.1	16.7	

It can be seen that 16.7% belong to the group in which the lateral side of the first phalanx is more than 10% longer than the medial and only 2.2% belong to the group with the medial side of the phalanges over 10% longer. Although the first phalanx of the hindleg is, on the average, shorter than that of the foreleg, the frequency of unequal growth in length is lower in the hindleg.

6. *Phalanx II.* The second phalanx in both normal and Ancon sheep ceases to grow after the fourth month; the diameter, however, continues to increase until maturity. Since the percentage increase in length is much less than normal, whereas the increase in width of the two breeds is the same, the adult bone is much shorter (24.4%) and wider (15%) than in normal sheep. The diameter-length ratio decreases with age in the normal and the reverse is found in the Ancon. This is also true for the first phalanx.

The frequencies of unequal growth on the two sides are as follows:

	Medial side over 10% longer	Normal range (<10%)	Lateral side over 10% longer	Total No. of bones
Newborn	1	32	2	35
2-month	0	7	0	7
4-month	0	4	3	7
6-month	6	8	0	8
Adult	1	21	2	24
Total No. of bones	2	72	7	81
Percentage of cases	2.5	88.9	8.6	

Again the asymmetrical second phalanges of the hindleg have a higher frequency on the lateral side but the incidence of asymmetry is much lower than in the first segment and in the same segment of the foreleg.

In both the fore- and hindlegs the cannon bones have, on the average, shorter lateral sides while the phalanges are longer. The crippling may intensify the situation, but the differences already exists in the newborn.

The above observations on the long bones lead one to conclude that the shortening of long bones can be caused by two means: (1) by slow growth as in the humerus, the femur and the first phalanx; (2) by complete arrest as in the radius, the metacarpus, the metatarsus, and the second phalanx of the hindleg. In general the second condition gives more shortening than the first. As will be discussed in a report

of a microscopic study, there are two kinds of epiphyseal plates in the long bones of the Ancon lambs. The epiphyseal plates in the humerus and the proximal end of the femur are hypoplastic, those in the distal end of the radius, the proximal end of the tibia and the distal end of the metacarpus are in various stages of disintegration, and those in the distal ends of tibia and metatarsus have grossly disappeared. The degree of shortening is, therefore, related to the activity of the cartilage.

III. Comparison of different segments

1. *Foreleg.* When the increase in length of the long bones in the foreleg of normal and Ancon males is plotted on a chart (Fig. 1), one can see that the bones of the 2-month old Ancon sheep, on the average, grow faster than normal, but that from 4-months on they lag behind the normal. This is especially so for the metacarpus. The single normal male specimen available for the 6-month stage happens to be smaller than the 4-month normal and 6-month Ancon sheep. It affects the picture of normal growth. It can be seen that length increase of the humerus in Ancons is very close to that of normal sheep, whereas growth in length of the radius-ulna and metacarpus are much retarded in Ancons.

The percentages of increase and retardation, together with our data on the indices for proximal/distal segments, which will not be given in this report, show that during postnatal growth there is a gradual decrease of growth in length and width from the scapula to the metacarpus, except that increase in length of the normal humerus fails to exceed that of the radius-ulna; on the other hand, the degree of retardation increases among Ancons in the same order, except that the degree of retardation of the humerus fails to exceed that of the scapula. In both instances the exceptional behavior may be due to the smaller size of the humeri in our normal specimens. The gradient, however, does not extend to the phalanges where intensity of growth increases and degree of retardation goes down again. But the second phalanx has a slower growth and higher degree of reduction than the first. It seems that the phalanges have their own gradient.

2. *Hindleg.* Fig. 2 shows that the increase in length of the long bones in the hindleg of the normal and Ancon males has the same general pattern as the foreleg, except that the normal femur has a greater

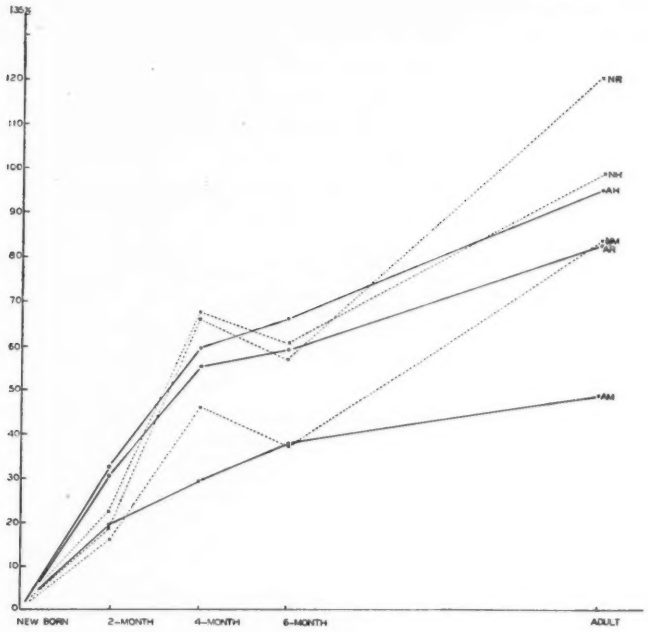

FIGURE 1.

Showing the percentage increase in length in the foreleg of normal and Ancon males. The value for normal adults is calculated from data for normal females and Ancon males and females.

Dotted line — normal
Solid line — Ancon
NH — normal humerus
AH — Ancon humerus
NR — normal radius-ulna
AR — Ancon radius-ulna
NM — normal metacarpus
AM — Ancon metacarpus

increase than the tibia, and the Ancon metatarsus fails to exceed that of the normal during the second month and has a strikingly low growth rate.

The percentages of increase and retardation, together with our unpublished data on proximo-distal indices, show that the hindleg represents a more typical gradient in length increase and retardation than does the foreleg, although they are generally of the same nature. Both

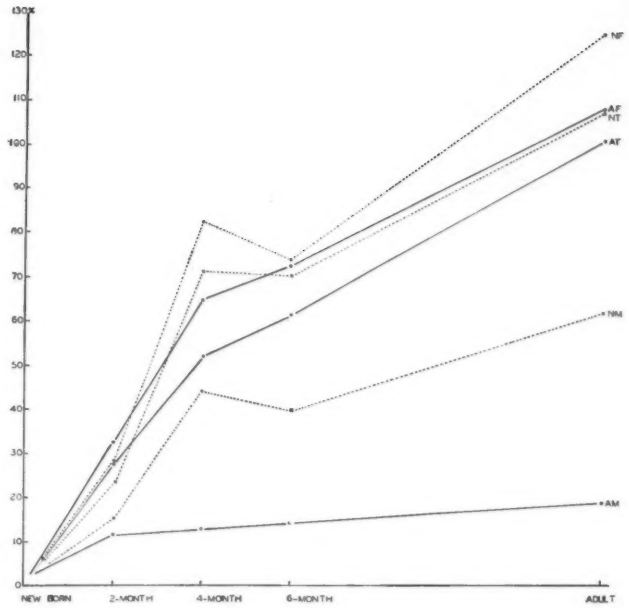

FIGURE 2.

Showing the percentage increase in length in the hindleg of normal and Ancon males. The value for normal adults is calculated from data for normal females and Ancon males and females.

Dotted line — normal
Solid line — Ancon
NF — normal femur
AF — Ancon femur
NT — normal tibia
AT — Ancon tibia
NM — normal metatarsus
AM — Ancon metatarsus

the fore- and hindlegs of the Ancon sheep, although showing some variation in retardation of segments, in general have the same gradient as the normal, and the rate of retardation is opposite that of the rate of increase. This gradient ends at the cannon bone and a new one arises from the first phalanx and declines again in the distal segments.

3. *Homologous segments.* The percentage retardation and our unpublished indices for homologous segments show that the hindleg is more retarded than the radius-ulna from the newborn to 6-month stages

but less so in the adult female. Since the percentages of increase and retardation in radius-ulna, as mentioned before, are irregular, this exception probably is due to variation in the radius-ulna rather than in the tibia.

E. Correlation between skeletal deformation and retardation

The skeletal deformations, which will be described in a morphological study, show that the specimens with vertebral abnormalities do not show occipital deformation, nor do any of them have the extremely shortened or crippled legs which are found in others. Only one case, specimen A20, had both the occipital and vertebral malformations, but its legs were not much shortened.

The abnormal curvature of the shaft and acute angle formation of the condyles in the Ancon femur also fail to show a correlation with crippling or with extreme shortening of the distal segments. The forelegs are usually more crippled than the hindlegs, yet the humeri are the least affected long bones; nor can the distortion of the femur be related to functional adaptation, since the hindlegs of sheep bear less body weight than do the forelegs.

When the leg bones in each individual are compared with the average length and the range of variation, it was found that one side may be more shortened than the other. The same situation is to be found in the homologous structures. In one 13 months old specimen the two metatarsi are of the intermediate type, but the other long bones are the longest among the Ancon material. With the exception of the extreme shortening of the medial second phalanx of the right hindleg, the other digital bones in this specimen are even longer than normal.

As the above observations failed to give definite information about the relative relationship of the bones to each other, the individual data for the leg bones of newborn male and adult female Ancon sheep were treated with Stockard's chart method (Fig. 3 and Fig. 4). The humeri were arranged in sequence from longest to shortest (A), the radii-ulnae (B) and metacarpi (C) then were arranged in the same order as the humeri in order to study the proximodistal relationship. Similarly, the femora were arranged from longest to shortest (D), then the tibiae (E) and metatarsi (F) in the same sequence. Similarly the sequence of radii-ulnae (H) were followed by tibiae (I), and that for the metacarpi (J) by the metatarsi (K). The results for newborn males are shown in Fig. 3 and those for adult females in Fig. 4. From these one may see the following:

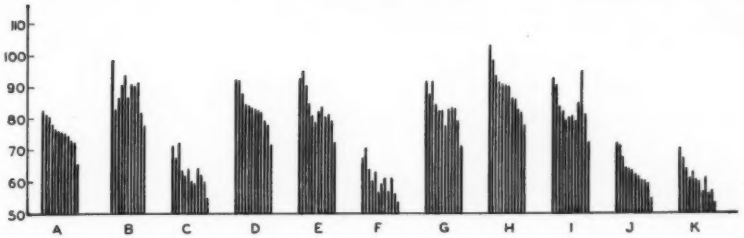

FIGURE 3.
Showing the correlation of length in the leg bones of newborn Ancon (male).

A. Length of humerus. Group of 12 humeri arranged in sequence from greatest to least length.
B. Length of radius-ulna. Same sequence as for humerus in A.
C. Length of metacarpus. Same sequence as for humerus in A.
D. Length of femur. Group of 12 femora arranged in sequence from greatest to least length.
E. Length of tibia. Same sequence as for femur in D.
F. Length of metatarsus. Same sequence as for femur in D.
G. Length of femur. Same sequence as for humerus in A.
H. Length of radius-ulna. Group of 12 radii-ulnae arranged in sequence from greatest to least length.
I. Length of tibia. Same sequence as for radius-ulna in H.
J. Length of metacarpus. Group of 12 metacarpi arranged in sequence from greatest to least length.
K. Length of metatarsus. Same sequence as for metacarpus in J.

The length of the newborn radius-ulna behaves irregularly relative to the length of the humerus. The situation in the adult is very irregular and no correlation seems to exist. The length of the newborn metacarpus, although also irregular, shows a definite correlation with the humerus, but the adults fail to give any indication of regularity. Length of the newborn tibia shows a better correlation with length of femur than does that of the adult. Length of the metatarsus in both the newborn and the adult behave irregularly in relation to length of the femur.

There is an irregular but definite correlation between length of the newborn humeri and femora, but in the adult the correlation is very weak, if present at all. Length of the tibia of newborn is in better accord with the radius-ulna than in the adult. Both the newborn and the adult metatarsi are in accord with the metacarpi, with slight irregularities. The length of the long bones, therefore, shows a better correlation in the newborn than in the adult. The adult radius-ulna, femur, and metacarpus, and to a lesser extent, the newborn radius-ulna, are very irregular.

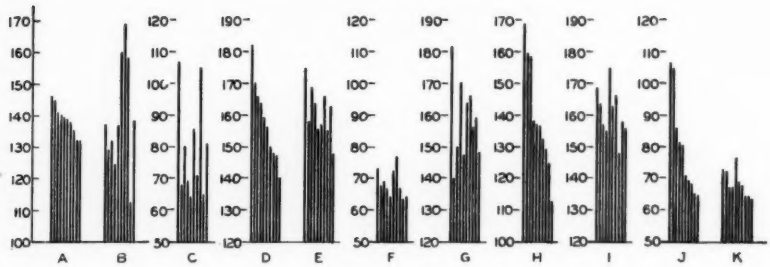

FIGURE 4.
Showing the correlation of length in the leg bones of adult Ancon (female).

A. Length of humerus. Group of 10 humeri arranged in sequence from greatest to least length.
B. Length of radius-ulna. Same sequence as for humerus in A.
C. Length of metacarpus. Same sequence as for humerus in A.
D. Length of femur. Group of 10 femora arranged in sequence from greatest to least length.
E. Length of tibia. Same sequence as for femur in D.
F. Length of metatarsus. Same sequence as for femur in D.
G. Length of femur. Same sequence as for humerus in A.
H. Length of radius-ulna. Group of 10 radii-ulnae arranged in sequence from greatest to least length.
I. Length of tibia. Same sequence as for radius-ulna in H.
J. Length of metacarpus. Group of 10 metacarpi arranged in sequence from greatest to least length.
K. Length of metatarsus. Same sequence as for metacarpus in J.

From Figs. 3 and 4 one can also see the range of length variation. The range in newborn is much more limited than in the adult. The humerus, the least affected bone, and the metatarsus, the most shortened one, have narrower ranges than the others.

Discussion

In regard to absolute size and percentage increase, the skeletons of our normal and Ancon sheep show a phenomenon that is generally encountered in sheep; that is, the female grows faster during the first two months than does the male. This is in agreement with Hammond and Appleton's (4) study of the Suffolk race and Schmitt's study of East Prussian races (12). The two month old Ancon sheep of both sexes show a greater percentage increase (not absolute size) than the normal. From the fourth month onward growth of the Ancon, in comparison with the normal, becomes more and more strikingly retarded.

According to Hammond and Appleton, among newborn lambs the weight of singles is 29% greater and that of triplets 9% less than twins. In our Ancon material, as one can see from the table in the previous section, seven out of eight newborn females were from twin births as contrasted with 4 out of 8 of the males. Among normal newborn lambs, there was only one twin out of three males and the one female was from a single birth. In the second month stage, both the normal female and normal male were from twin births; one of the two Ancon females was a triplet, while the two males were singles. From the situation in the newborn Ancons, one would expect to have smaller size and greater percentage retardation in the newborn females than in the males; the results show the opposite to be true. In the case of the second month, the aforesaid difference in percentage increase between normal and Ancon sheep might be due to the effect of the distribution of animals between single and multiple births. The material seems insufficient to evaluate the effect of multiple births on the percentages of increase and of retardation.

Todd and Wharton (14, 15) have shown that the cranial parts of sheep develop first and the facial skeleton later. Our material shows the same phenomenon. Hammond and Appleton found that early maturing skulls are shorter and broader. The Ancon skull is reduced in length, but not in cranial width and height. The facial width and height are more retarded than the respective measurements of the cranium. The differences in rate of retardation show the Ancon skull to be shorter and broader than normal, hence resembling more the early maturing type than the later maturing. Furthermore, the late developing parts in the skull, which have higher postnatal growth rates, are subject to greater retardation. The occipital region, especially the ventral side, is normal in width, but retarded in length. It is, however, frequently malformed, in spite of the fact that it is a structure with an active early development and stagnant postnatal growth.

The percentage increase in the vertebrae of sheep depends upon their regional characteristics; for instance, in the cervical vertebrae it is greater for length, in the thoracic for height, and in the lumbar for width and thickness of centrum. The sacrum is subject to sex dimorphism in that the female has a greater percentage increase in width and the male in height. The vertebrae of Ancons are not affected in length but they are retarded in height, width, and thickness of the centrum.

In human chondrodystrophy the neck and head of the humerus (or femur) are short, whereas its tuberosity (or trochanter) is more prominent (Maa's study cited by Dietrich, 2). The indices on head-neck divided by tuberosity (or trochanter) of the humerus (or femur), which are not given in this report, show that the tuberosity and trochanter in the Ancon sheep, contrary to the finding in human cases, are relatively more retarded than the head-neck.

Huxley (6, 7), using Hammond's weight measurements of the appendicular bones of sheep, first demonstrated negative allometry in the growth of sheep limbs. He omitted the radius-ulna due to its irregularity. The growth rate in our normal and Ancon limbs are in accord with his observation of negative allometry but the normal radius-ulna again failed to fit the gradient. Brovar and Leontjeva (1) confirmed Huxley's results and further demonstrated a rise of the gradient in the phalanges. The measurements in the phalanges of our material are in agreement with their results; that is, the growth rate of the leg bones declines proximo-distally to be lowest in the cannon bone, but rises in the first phalanges and decreases again distally. The rate of retardation in the limbs is opposite to the gradient of growth.

Hammond and Appleton have emphasized that, in addition to the shorter and broader skull, the domesticated and highly selected early maturing breeds have narrower vertebrae, wider scapulae, and shorter but thicker leg bones than the semi-wild or late maturing sheep. The actual size of our normal specimens shows that our newborn of both sexes lie between Hammond and Appleton's ram and ewes of newborn Suffolk and that our adults are all shorter than theirs. As the Ancon sheep has a broader and shorter skull, relatively wider scapula, narrower vertebrae and shorter leg bones, it seems to present an extreme expression of early maturation. This is especially true for the cannon bone which is the most retarded one in the Ancon skeleton. However, this does not mean that the retardation is related to sex or thyroid hormones. It is impossible to assume that sex hormones have exerted a maturing effect in the newborn lambs in which some of the epiphyseal plates have completely disappeared. Moreover, the differential growth of the cannon bones and digits, the malformation of the occipital region, etc., are characteristic manifestations of chondrodystrophy rather than domestication, high selection, or the general effect of hormones. That endocrine glands have no relation to chon-

drodystrophic dwarfism has been demonstrated for humans (2, 11), dog (13) and Creeper fowl (8, 9, 10). Ancon sheep are, therefore, not an exceptional case. Our observations on thyroid glands show normal and congenitally hypoplastic glands (the latter probably due to iodine deficiency) in both normal and Ancon sheep ruling out the possibility of an influence of the thyroid gland on the Ancon condition.

As the hindlegs are more retarded than the forelegs and the distal segments more than the proximal ones, the mode of reduction has more similarity to that of the Creeper fowl than to the chondrodystrophy of human beings and other animals.

Summary

1. The skeletons of 14 heterozygous (normal) and 50 homozygous (Ancon) sheep from the Norwegian stock were used for this study.

2. In general the growth rate of sheep is higher in females than in males during early stages, but lower after the fourth month. With the exception of the metatarsus, the relative growth of Ancon long bones exceeds that of normal sheep during the first two months of life, but subsequently the former lags behind the latter.

3. The facial skeleton shows a greater percentage increase than the cranium during postnatal life. The Ancon skull is retarded in total length and to a lesser extent also in cranial length, but it is normal in cranial width and height. The facial region has a greater retardation than the cranial region. Similar to the skull proper, the mandible is also reduced in length but is normal in width.

4. The percentage increase in the vertebrae between birth and maturity varies according to their individual characteristics; it is greater for length in the cervical, for height in the thoracic, and for width and thickness of the centrum in the lumbar vertebrae. In Ancon sheep the vertebrae as a whole are only slightly retarded in length but much reduced in height, width and thickness of the centrum, particularly in the thoracic and lumbar regions.

5. The ribs show more retardation in width than in length. The transverse diameter of the thoracic cavity, in comparison to its depth, increases with age in normal sheep and more strikingly so in Ancons. The depth is reduced in Ancons but the transverse diameter is nearly normal.

6. The increase in length of the leg bones shows negative allometry. The rate of retardation is opposite to the rate of increase, that is it becomes more extreme proximo-distally. The growth gradient shows a second maximum in the first phalanx and then falls again distally.

7. The actual diameters of the humerus and the femur of Ancon sheep are smaller than normal in all stages. Those of the tibia and metatarsus are wider in the newborn but gradually become narrower with age. Those of the radius-ulna, metacarpus, and phalanges are wider in all stages. Relative to their length, all leg bones of Ancon sheep are wider than normal, except for the humerus which is slightly narrower.

8. The pelvis of Ancon sheep shows more reduction in length than in width, and the transverse diameter is slightly wider than normal.

9. The hindleg is more retarded in length than the foreleg.

10. The newborn males and adult females of Ancon sheep were analysed by Stockard's chart method. This revealed that in newborn animals the sequence of shortening of one segment shows a closer relation to the degree of shortening in other segments than in adults. The radius-ulna, metacarpus and femur in the adults are very irregular.

11. There is a medio-lateral difference in the retardation of length of cannon bones and digits. There are more cases of lateral shortening in the former and more of medial shortening in the latter. This situation is more severe in the foreleg than in the hindleg.

Acknowledgments

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MORPHOLOGICAL STUDY ON THE SKELETON OF ANCON SHEEP

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Ancon sheep are disproportionately dwarfed sheep which transmit this trait to their progeny as a recessive character. It first occurred in 1791 at Dover, Massachusetts, and became extinct at about 1876 (10, 22). In 1919 another short-legged lamb appeared in the flock of a Norwegian farmer (51). The history and genetics of these two independent mutations will be reviewed elsewhere.

As the manifestation of the mutation is a disproportionate dwarfism, characterized by a decrease in length and increase in diameter of the long bones as well as an enlargement of the joints, Ancon sheep have long been assumed to be achondroplastic: no Roentgenological or histological study has appeared in the literature.

The term achondroplasia was originally given by Parrot (38) in 1876 to certain fetal dwarfs. Kaufmann (23) in 1892 replaced the term with chondrodystrophy because the dwarfism is not due to the absence of cartilage but to the inactivity of cartilage that is present. Chondrodystrophy in humans caused a wide and lasting interest, which has resulted in a tremendous and confusing literature (16, 34). In mammals hereditary dwarfism has been known in cattle (bulldog calf of Dexter breed (13, 41), Telemark breed in Norway (33, 52), African cattle (11), different races of dogs (46), rabbits (8), South Down sheep (6), goats (17), guinea pigs (33), and pigs (33, 34)). Inherited shortening of long bones in the leg is also known in turkeys (2), pigeons (21), and salamanders (31). Chondrodystrophy in chickens, e.g. the Creeper fowl, has been thoroughly studied by Prof. Landauer and his collaborators (24, 25, 27, 28).

This Department obtained one Ancon and two heterozygous ewes from Norway in 1927. Since then the stock has been maintained by

inbreeding and occasional outcrossing to Cheviots. Skeletons from both the heterozygous and homozygous animals were accumulated over a period of years and are the material used in the present study. The present communication deals with morphological abnormalities; data on the growth of the skeleton of Ancon sheep will appear in a separate report.

Material and Methods

Fifty homozygous (Ancon) and fourteen heterozygous (normal) skeletons of newborn, 2-month, 4-month, 6-month, and adult stages were used for this observation. The whole skeletons of those individuals younger than 2 months and the appendicular skeletons of all ages were preserved in formalin, while the axial skeletons and pelves of sheep older than 4 months were preserved dry.

One fore- and one hindleg from each of the four stages of normal and Ancon specimens were bisected frontally for a study of the fusion of the epiphysis with the metaphysis and the formation of the trabecular system. They were soaked in 1-2% potassium hydroxide for 1-3 weeks, thoroughly washed with hot water, and dehydrated up to 70% ethyl alcohol. For examination of the thickness of the shaft cross sections of the middle of the shafts were made. A middle piece, about 3 mm. in thickness, of the distal end of the radius was sawed out from each end of the above frontal bisections for histological examination. The proximal end of the radius was also taken from two Ancon and one normal lambs for comparison. Since many Ancon metacarpi show differences in the size of the medial and lateral condyles, the distal halves from two newborn metacarpi were sectioned. They were all refixed in Heidenhain's susa, decalcified in 3% hydrochloric acid in 70% alcohol, imbedded in celloidin, stained in hematoxylin-eosin or azan. Only one case had half of the distal end of the radius sectioned serially. Ground sections of the middle of shafts of metacarpi from both normal and Ancon adults were made for a study of the differences in the Haversian systems.

Observations

A. Macroscopic abnormalities

a. *Bone texture.* The skeleton of the male is usually thicker and rougher with more pronounced spines, crests and processes than in

the female. This condition is greatly exaggerated in skeletons of Ancon sheep, especially in the older appendicular bones. Exostosis-like structures (Fig. 2, D, E) appear for the first time in 2 month Ancon specimens. In addition to extramembraneous ossifications (sesamoid bones) in the joint capsule and along the tendon and tendon sheath, fusion of vertebrae by massive ossifications of vertebral ligaments are common to the adults of both breeds. Those in the Ancon skeletons seem to appear earlier and are more extensive. This is especially so in the leg bones of crippled animals. Three cases in our material show nodular formation in the costal cartilage: one is an 11-year old normal female and the other two are 8-year old Ancon females. Such nodular ossification probably is unrelated to the chondrodystrophy in sheep.

b. *Occipital region.*

1. *Position of occipital condyles.* In Ancons ventral distance between the two occipital condyles is much less than normal. It varies from a narrow intercondyloid incisure to complete fusion of the two condyles. The dorsal distance is in general more normal than the ventral. One case (specimen A20, 2½ months female) has a complete fusion dorsad as well as ventrad; this results in a concave ring around the much narrowed foramen magnum and articulation with the similarly deformed atlas. The right condyle sometimes is more horizontally or dorsally displaced than the left, so that the form of the foramen magnum is distorted and the angle and distance between the two condyles are changed. A few cases show atrophic condyles.

2. *Depression of occipital surface.* In the skull of newborn Ancon sheep the occipital surface at the junction of the squamous and the lateral occipital is normally cartilaginous and convex. The skulls of newborn Ancons may show various degree of concavity at this cartilaginous junction, possibly due to abnormal ossification. The dorsal lip of the foramen magnum in the more abnormal cases protruded backward; the whole margin may be tubular and turned backward instead of the normally downward direction.

3. *Size of foramen magnum.* When the condyles come close to each other at the ventral side, the diameter of the foramen magnum becomes proportionately smaller. Sometimes it is so small in Ancon sheep that the spinal cord can barely pass through. As mentioned before the position of the condyles will change the form of the foramen.

4. *Occification centers* in the basi-occipital. There are three cases in the Ancon material which show extra ossification centers at the basi-occipito-sphenoid junction. Two of the three cases show one side of the basioccipital underdeveloped and shorter than the other.

5. *Occipito-parietal region.* By taking a dorsal view of the occipito-parietal region, the right side in some specimens is not as eminent as the left.

The above abnormalities occur in 24 of 38 skulls. The legs of most of these specimens show an intermediate degree of shortening, but extreme types also occurred.

c. *Vertebral column.* As has been mentioned before, fusion of vertebrae, especially in the lumbar region, by callus-like formation at the ventral surface of the vertebral body are common to aged skeletons of both breeds. The following abnormalities are found only in Ancon skeletons. Since there are only a few cases, a brief description of each is given below:

Specimen A10, female, 5 2/3 years old, has the centrum of the lumbar vertebrae more flattened than normal and their transverse processes atrophic.

Specimen A41, female, 3 years old, has the right transverse processes of the lumbar vertebrae narrowed, shortened, and bent ventrally, except the process of L_7 which on the contrary is widened to articulate with the pelvis.

In specimen A36, male, newborn lamb, vertebrae T_{11}-L_5 zigzag due to bilaterally unequal length of centra (scoliosis), the vertebral canal is triangular, one side is smaller than the other due to unequal size of the neural arches.

Specimen A48, male, newborn lamb, has the right transverse processes of L_7-S_1 fused together and articulated with pelvis, the left process of L_7 vestigial, joint of $L_{6\text{-}7}$ shifted toward the left, and the centrum of S_1 shorter at the right side (scoliosis).

Specimen A20, female, 2½ months old, has the articular surface of the atlas deformed to match the ring-formed occipital condyles, the axis has no dens, anterior end of vertebral canal of the axis is constricted, spinous processes and neural arches of the lumber vertebrae are fused, $L_{5\text{-}6}$ bulges toward the abdominal side (lordosis), and $S_{3\text{-}4}$ curves toward the right side (scoliosis).

With the exception of A20, the skulls of the above mentioned specimens have no abnormalities in the occipital region.

d. *Curvature and condyloid angle of femur.* (Fig. 2, D, E). In newborn the femurs normally possess a slight curvature centered in the middle of the shaft. It increases with age. The curvature in the femur of Ancon sheep may be noticeably larger, bending backward with the center either at the lower half or one third of the shaft. Bones with extremely acute angles are usually accompanied by a greater curvature. The angle formed by the shaft and condyles normally varies from 90° to 100°. In Ancons, due to retroproximal shifting of the condyles, the angle may vary from slightly less than 90° to very acute. The longitudinal axis of the femur is normally perpendicular to the condyloid surface, but in the extreme Ancon cases they may be parallel to each other (E). We failed to observe any abnormal curvature in the femur of the newborn Ancon sheep. The angle in some of them, however, is less than 90°, which may be the beginning of deformation. There are two cases each in 2-month and 4-month Ancons that have a condyloid angle of 45°.

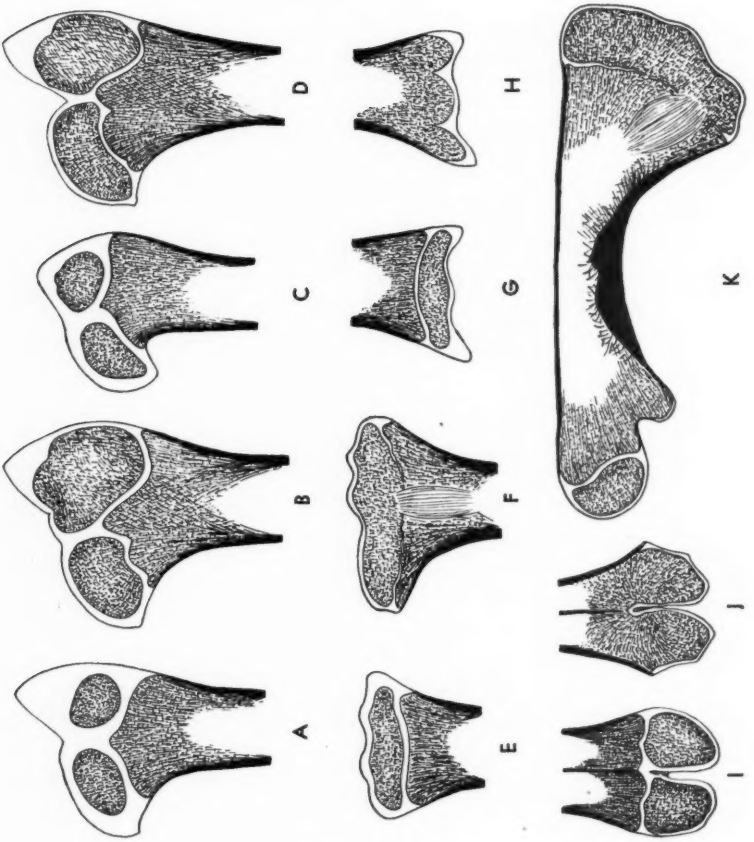
When the femur is crooked the position of the patella, normally anterior, is shifted to one side with a new articular surface. The original facet which normally faces anteriorly now faces downward and is partially shared for articulation with the tibia. The articular surface of both the femur and tibia are, in such cases, very much enlarged with many exostosis-like formations (D).

e. *Enlargement of metaphysis* (Fig. 2, A, B, C, D, E). The distal end of the femur, radius, cannon bones, both ends of tibia, and to a certain extent the phalanges are enlarged to various degrees; usually in proportion to the degree of shortening of these bones. The bones of newborn show a sharply defined enlargement at the metaphyseo-epiphyseal junction. To a lesser extent the junction of the heads of humerus and femur are slightly larger than the normal ones.

f. *Flattening of bone shaft* (Fig. 2, A′, C′). The radius and metacarpus of Ancon sheep are more flattened and the ulna is more laterally located than in the normal bones.

B. Frontal sections of long bones

a. *Fusion of epiphysis with metaphysis.* Although our material was not prepared for a study of the sequence of epiphyseal fusion it offered interesting information on the differences in the epiphyseal growth between the two kinds of sheep. In the normal foreleg the


A
B
C
D
E
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G
H
I
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K

FIGURE 1.

Semidiagrammatic drawings showing the establishment of permanent architecture of trabecular systems and their modifications in the long bones of normal and Ancon sheep.

A. Proximal end of humerus of normal male fetus (near term) A43, showing simple V-shaped curvature of epiphyseal plate with straight and parallel trabeculae.

B. Proximal end of humerus of newborn Ancon female B22, showing double V-shaped curvature of epiphyseal plate with peripherally fan-shaped and centrally cross-laced trabeculae, the permanent architecture.

C. Proximal end of femur of normal newborn female B26, showing simple V-shaped curvature of epiphyseal plate with straight and parallel trabeculae.

D Proximal end of femur of newborn Ancon female B22, showing double V-shaped curvature of epiphyseal plate with peripherally fan-shaped and centrally cross-laced trabeculae, the permanent architecture.

E. Proximal end of tibia of normal newborn female B26, showing shallow U-shaped curvature of epiphyseal plate with fan-shaped trabeculae (primitive type remains to be permanent).

F. Proximal end of tibia of newborn Ancon female B22, showing disintegrating epiphyseal plate, centrally perforating canal with long and straight (intramembraneous) trabeculae, and horizontal extension of trabeculae to the enlarged metaphysis (right side).

G. Distal end of tibia of normal newborn female B26, showing shallow U-shaped curvature of the epiphyseal plate with fan-shaped trabeculae.

H. Distal end of tibia of female fetus (near term) of Ancon A42, showing the disappearance of the epiphyseal plate and the presence of meshwork trabeculae.

I. Distal end of metatarsus of normal newborn female B26, showing shallow U-shaped curvature of epiphyseal plates with fan-shaped trabeculae.

J. Distal end of metatarsus of newborn Ancon female B22, showing the disappearance of epiphyseal plates and the radiating trabeculae developed from the cartilage in the roof of the sagittal notch, cross-laced with the trabeculae developed from the articular cartilage.

K. Sagittal section of femur of newborn Ancon male A46, showing the disintegrating epiphyseal plate with perforating canal at the posterior half of the plate, and thickening of the shaft in the posterior wall with a few radiating trabeculae.

A
A'
B
B'
C
D
C'
D'

FIGURE 2.

Showing the length and thickness of the shaft of long bones of the fore-and hind-legs (and curvature and condyloid angle of femur). Approximately x 0.23.

A. Long bones of the right forelegs of newborn lambs. These are arranged from left to right, in three groups of 4 bones each. In each group the first bone is normal, then follows the longest, intermediate and shortest of the Ancon bones. The first group of 4 is the humerus, second the radius-ulna and third the metacarpus. Notice that the longest Ancon metacarpus is shorter than the average normal; note also the development of the metaphyses of Ancon radii-ulnae and metacarpi and the unequal development of the metacarpal condyles, especially the lateral (left) side of the last Ancon metacarpus in the picture.

A′. Cross sections through the middle of the shafts of left humerus, radius-ulna, and metacarpus, upper row normal and lower row Ancon. Notice the slight thickening, lateral shifting of the ulna, and flattening of the metacarpus in the newborn Ancon.

B. Long bones of the right hindlegs of newborn lambs, arranged as for the foreleg. Notice that the longest tibia and metacarpus of the Ancon sheep are shorter than the average normal; note also the enlargement of the lower metaphyses of the Ancon femur, tibia, and metatarsus and the slightly unequal development of the condyles in the latter.

B′. Cross sections through the middle of the shafts of left femur, tibia, and metatarsus, arranged as in A′. Notice the thickening and flattening of the newborn Ancon bone.

C. Long bones of the right forelegs of adults, arranged as in A. Notice the severe shortening of the radius-ulna and the metacarpus, the enlargement of the metaphyses and the irregular development of the metacarpal condyles of the Ancon bones.

C′ Cross sections through the middle of the shafts of the left humerus, radius-ulna and metacarpus in the adult, arranged as in A′. Notice the thicker normal and flatter Ancon bones.

D. Long bones of the right hindlegs of adults, arranged as in A. Notice the enlargement of the metaphyses and the severe shortening of the metatarsus in the Ancon bones.

D′. Cross sections through the middle of the shafts of the left femur, tibia and metatarsus in the adult, arranged as in A′. Notice the thicker normal and flatter Ancon bones.

E. Lateral view of femora showing the curvature of the shaft and the condyloid angle. From left to right, the first femur is normal bone, the other four are the Ancon bones in various degree of deviation in curvature and angle.

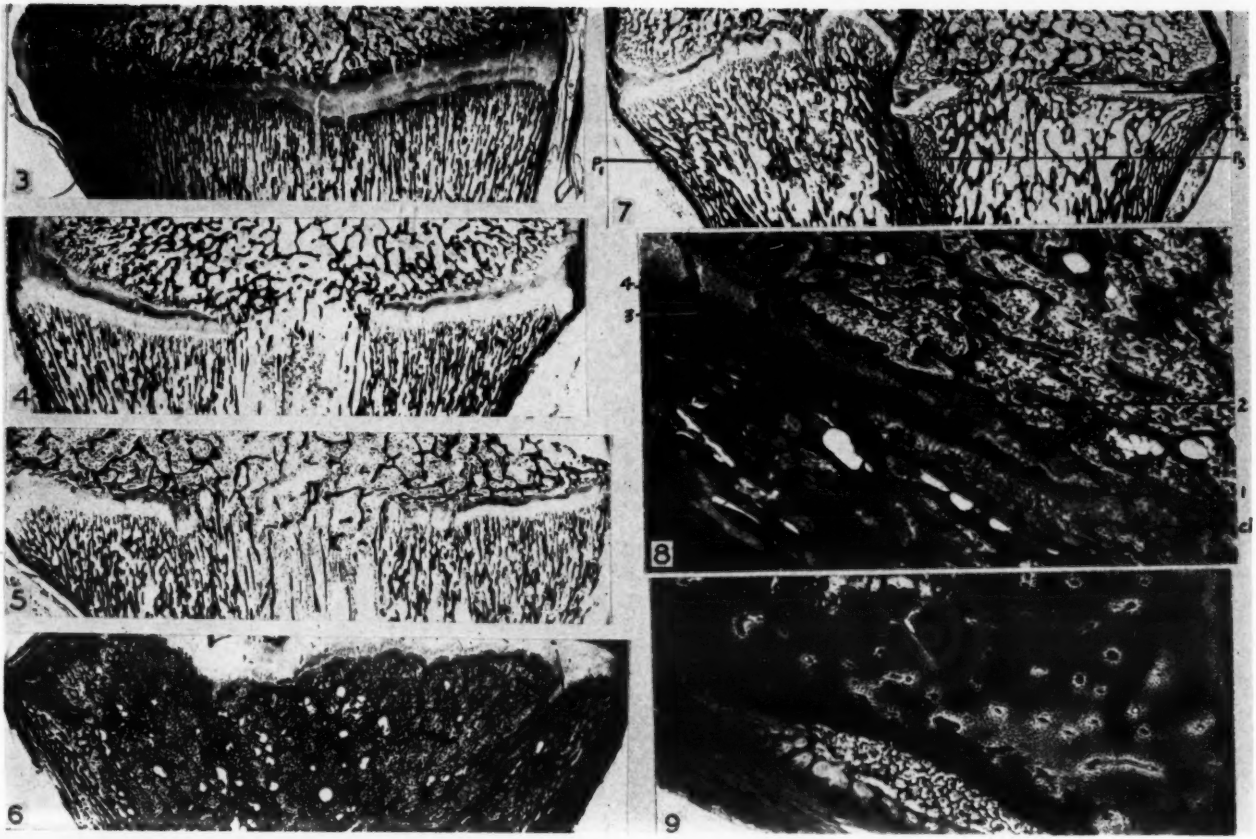
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FIGURE 3.

Longitudinal section of the distal end of the radius in a normal newborn lamb showing the thickness of the epiphyseal plate, the perforation in the articular and epiphyseal cartilage, and the density of the primary, cancellous and epiphyseal trabeculae. Hematoxylin-eosin × 4.8.

FIGURE 4.

Longitudinal section of the distal end of the radius in a newborn Ancon lamb showing the practically normal thickness of the epiphyseal plate, the perforation in the articular and epiphyseal cartilage, the presence of a perforating canal with long and straight trabeculae (intramembraneous origin), and relatively fewer and shorter primary and cancellous trabeculae, and slightly looser epiphyseal meshwork. Azan × 4.8.

FIGURE 5.

Longitudinal section of the distal end of the radius in a newborn Ancon lamb showing much thinner epiphyseal plate, larger perforating canal, scarcer and shorter primary and cancellous trabeculae and much looser epiphyseal trabeculae.

FIGURE 6.

Longitudinal section of the distal end of the radius in a newborn Ancon lamb showing the severely disintegrated epiphyseal plate oblique in position, parallel to the metaphyseal surface, and the meshwork of the trabeculae. Hematoxylin-eosin × 4.8.

FIGURE 7.

Longitudinal section of the distal end of the metacarpus in a newborn Ancon lamb showing (1) difference in position and size of the perforating canal of the medial (left) and lateral (right) epiphyseal plates, (2) difference in the density of the primary, cancellous, and epiphyseal trabeculae on the two sides, and (3) the thinner periosteal bone on the medial side as compared with the lateral. 1 — pericanal, 2 — intermediate, 3— "Perioststreifen," and 4 — marginal regions described in the text. Notice the presence of horizontal bone spicules on the lateral side. p1—periosteal bone on the medial side. p2 — periosteal bone on the lateral side. p3 — intramembraneous bone from the fibrous septum. Azan × 4.8.

FIGURE 8.

Enlarged view of the epiphyseal plate shown in the upper right corner of Fig. 11, demonstrating 4 regions (1, 2, 3, 4) and cartilaginous islands (ci). Notice the perforating blood vessels in the epiphyseal plate originating from the articular cartilage and the epiphyseal marrow cavity. Hematoxylin-eosin × 16.

FIGURE 9.

Section through the peripheral region of the epiphyseal plate shown in Fig. 6. Notice the diaphyseal surface of the epiphyseal plate facing the metaphyseal surface, the formation of the conical clumps and the effect of blood vessels on the articular and epiphyseal cartilage. Hematoxylin-eosin × 18.

10
11
12
13
14
15

FIGURE 10.

Magnification of a portion of Fig. 9 showing the effect of the blood vessel on the size of cartilage cells and on the staining of the matrix. Hematoxylin-eosin × 92.

FIGURE 11.

Magnification of the normal epiphyseal plate of Fig. 3 showing the effect of the blood vessel on the size of the cartilage cells and on the arrangement of cell columns. Notice the thickness of the plate. Hematoxylin-eosin × 88.

FIGURE 12.

Marginal region of the lateral epiphyseal plate of the metacarpus shown in the right corner of Fig. 7, showing the outward extension followed by inward extension of cell columns. Azan × 88.

FIGURE 13.

Magnification of the intermediate region from the same block but different section of Fig. 5 showing the structure of the conical clumps. Notice the thickness by comparing with Fig. 11. Hematoxylin-eosin × 93.

FIGURE 14.

Transverse ground-section of the middle of metacarpal bone of a normal adult (female) showing the structure and arrangement of the Haversian systems. Silver impregnation × 82.

FIGURE 15.

Transverse ground-section of the middle of metacarpal bone of an Ancon adult (female) showing the structure and arrangement of the Haversian systems. Notice the irregularity and scarcity of complete Haversian systems. Silver nitrate impregnation × 82.

proximal epiphysis of the radius was fused and the distal epiphysis of the humerus was in the process of fusion during the fourth month and the epiphysis of the metacarpus begins to fuse during the sixth month. In the Ancon the proximal and distal ends of the humerus and proximal end of the radius are grossly normal except for their thinner appearance (Fig. 1, B). In our newborn material some of the distal epiphyseal plates of the radius (Fig. 4, 5, 6) and the metacarpus (Fig. 7) were poorly perforated and layers of only a few millimeters thickness were left at the periphery. The remaining epiphyseal plates were often oblique instead of horizontal. They formed funnel-like structures in which the ossification surface faced outward and parallel to the enlarged metaphyseal surface. The structure of the epiphyses in Ancon sheep therefore depends upon the degree of chondrodystrophy of the animal and of the particular bone used for study. The conditions observed in the newborn Ancons are at times also present in later stages. Some of them are normal as late as the 6-month stage. These are, however, thinner due to ageing. The variability found in the foreleg also occurs in the hindleg.

The epiphyseal plates of the distal ends of the tibia and metatarsus are still present during the fourth month in normal sheep, but disappear by the sixth month, while the rest of the epiphyseal plates are still active. In the hindleg of the newborn Ancons, which we examined with hand lens, only the proximal epiphyseal plate of the femur was normal (Fig. 1, D). Its distal epiphyseal plate was poorly perforated. There were a large canal and fragmentary cartilaginous islands at the posterior side of the plate and the anterior part was thick and long (Fig. 1, K). The proximal plate of the tibia was similarly perforated and fragmentary (Fig. 1, F); its distal plate was not visible with the naked eye (Fig. 1, H). The epiphyseal plate of the metatarsus was as degenerate as that of the distal tibia but usually retained a small amount of the original articular cartilage lodged in the roof of the sagittal notch (Fig. 1, J).

According to Hammond and Appleton's citation (18), epiphyseal fusion in the foreleg occurs in the following order: distal humerus, proximal radius, metacarpus, distal radius and proximal humerus. The order of fusion in the hindleg is: distal tibia, metatarsus, proximal femur, distal femur, and proximal tibia. Our normal material has the same sequence.

b. *Trabecular system* (Fig. 1). The permanent type of trabecular system was already established at birth in the humeri but this was not the case with all of the femurs. The appearance of the permanent system of trabeculae in the humerus is essentially the same as in the femur (A-D).

The arrangement of the trabeculae has a direct relation to the curvature of the epiphyseal plate. In the proximal end of the humerus (or femur), the plate, in early stages, is in the form of a very shallow inverted "U". Upon the differentiation of the neck and trochanter (or tuberosity), the curvature is at first changed to an inverted "V" (A, D) and then to a double "V", linked together in the middle (B, D). The epiphyseal plates of the other long bones generally persist in a primitive form, i.e. a shallow U-shape (E, K). When it is in the latter form, the trabecular system is invariably straight and parallel, or fan-shaped, following the expansion of the metaphysis. By changing the inverted "U" to a double "V" the dome-shaped permanent system is established (B, D). This formation seems to be the result of the trabeculae forming along the course of the blood vessels which are normally perpendicular to the surface of the plate. The vessels are perpendicular to the surface of the epiphyseal plate because they extend from the marrow cavity into and through the eroded cell-columns of cartilage. The latter are at right angles to the plate. When the curvature is in the form of double "V", there are two arms of the "V" facing each other in the median line. When the trabeculae are perpendicular to these two arms the system will eventually become "cross-laced."

In cases in which the epiphyseal plates in Ancon sheep are complete but less active than normal, the trabecular form is not changed but the texture is looser and has more pronounced horizontal bars. This, to a certain extent, decreases the distinctness of the fan- and dome-shaped appearance. Since the proximal-epiphyseal plates of the humerus and femur of Ancon sheep are complete but hypoplastic there is no change in the form but only in the denseness of the system. When the cartilaginous plate is highly perforated and disintegrated and a large canal connects the epiphyseal and diaphyseal marrow cavities long and parallel trabeculae without horizontal bars are present along the blood vessels (F, K). These are resorbed in the adult bone. When the plate is completely fragmented by the blood vessels, the trabeculae form a meshwork which, since it

originated from the slow growing articular cartilage, lacks a definite pattern: (H) The most striking phenomenon occurs in the metarsus. In the newborn Ancon this bone has lost both the medial and lateral plates but an oval piece of cartilage remains in the center to form additional trabeculae. These trabeculae are not parallel and fan-shaped but instead radiate out from this cartilage and mesh with trabeculae formed from the articular cartilage (J). These findings further indicate the importance of the epiphyseal curvature for the development of the trabeculae.

c. *Cross sections of shaft* (Fig. 2, A′, B′, C′, D′). Cross sections through the middle of the shafts of long bones revealed that the Ancon bones were thicker than normal in the newborn (A′, B′) but thinner in the adult (C′, D′). This phenomenon is more conspicuous in the hindlegs. Even in considering the retardation in length these shafts in the adult Ancon are still relatively thicker than normal. It is also interesting that the shaft of the bent femur is not thickened.

C. Microscopic structures

The normal structure of the epiphyseal plate is well known to microscopic anatomists, therefore no description seems necessary. However, a few points which are important for a comparison with the chondrodystrophic cases should be mentioned here.

a. *Normal epiphyseal plate* (Fig. 3, 11). Many nutrient vessels penetrate into the articular cartilage in which they branch and anastomose before entering the epiphyseal marrow cavity. The vessels along the junction of the epiphyseal and articular cartilage always send side branches through the plate into the diaphyseal marrow cavity. Along these, connective tissue and embryonic cells form what in the German literature is called the "Perioststreifen." In addition, some small thin-walled vessels (largest found to be 166μ, mostly 50-80μ) go from the epiphyseal marrow cavity to that of the diaphysis. There is little connective tissue along these vessels and they usually pass straight through the plate without branching. When the vessel is branched the cartilage lying between the branches is cone-shaped; with the wider base usually facing the diaphyseal side. Inside of the cone the normal components of this region (proliferating, flattened and vesicular cells) are present and the arrangement is in the normal order. However, the cells nearest to the blood vessel are smaller and

flattened along its longitudinal axis. Imbedded in the adjacent matrix are the coarser fibers. Both the perivascular matrix and the fibers take a deeper red stain in hematoxylin-eosin and a deeper blue in Mallory or azan preparations than does the rest of the matrix. The color intensity in the perivascular region, therefore, is similar to that found in the proliferating zone. Lodged in the deeper stained matrix are one or two layers of flattened cells, as has just been mentioned. Next to this layer, in the hypertrophied zone, is a row of vesicular cells smaller in size and parallel to the course of the blood vessel. Outside of this both the flattened and hypertrophied cell-columns lose their straightness and continuity. In sections these irregular cell columns are spindle or oval-shaped with a single row of cells at the ends and two or three rows in the middle. The above mentioned perivascular structure is the same as that in the Ancon bone, but in the latter there are more and larger perforations and anastomoses (Fig. 8, 9, 10).

The thickness of the proliferating zone is 330-420μ, of the flattened cell column 330-420μ (or 35-40 cells), and of the hypertrophied column 110-190μ (or 7-12 cells). The primary bone spicules vary from 160-250μ in length. The trabeculae of the cancellous bone are about 830μ in length and 100-160μ in width. The periosteal bone reaches the margin of the plate in a single thin layer. The epiphyseal plate is curved toward the metaphyseal side like a shallow and inverted "U" with thin arms in which are the greatly shortened cell columns.

b. *Abnormal epiphyseal plate.* In the newborn or intermediate stages the distal plate of the Ancon radius may be normal or exhibit various degrees of disintegration. Usually a bone with normal length has also a normal epiphyseal plate microscopically.

1. *Mild degree of hypoplasia of the distal epiphysis of the radius* (Fig. 4). There is a large eccentrically located perforating canal (4-5 mm.) connecting the two marrow cavities in which are long and straight bony spicules of intramembraneous origin. The remainder of the plate is fairly even in thickness and cellular structure. There are more perforations (diameter 50-80μ) and anastomoses around the canal and here more cone-shaped clumps are found. The proliferating zones with multicellular capsules are 130-200μ thick, the flattened cell column 160-200μ (or 15-25 cells), and the hypertrophied column 80-110μ (or 6-7 cells). Most of the columns are not straight but interrupted into many patches each containing 2-3 rows of cells. The

length of the primary trabeculae is moderate (160-320μ). They are composed of cartilaginous bars and newly formed bone on each side. The cancellous trabeculae are thick but fewer and shorter than normal and are more longitudinal (80-150μ) than perpendicular (50-100μ); all have some cartilaginous remnant. Osteoblasts are abundant and mostly epitheloid in form. Osteoclasts are few in number. Periosteal bone reaches the epiphyseal plate in a single layer with many subperiosteal buds.

2. *Intermediate degree of hypoplasia of the distal epiphysis of the radius* (Fig. 5). The perforating canal is larger (8 mm.) than normal and the remaining plate is thinner with local variations in thickness and structure. The epiphysis can be arbitrarily divided into four regions from the inside toward the periphery (Fig. 8): (1). The region surrounding the canal is also perforated, and often separated into islands of different sizes, each surrounded by ossified bony plates. The fragmentary cartilaginous islands are derived from the cone or cones inside of which are the flattened and hypertrophied, but not vesicularized, cells. Proliferating cell-types may occasionally be present. (2). An intermediate region, outside of the first, is relatively more active. The proliferating zone is thin (110-130μ) and interrupted by blood vessels. Each capsule in this zone usually contains a single cell. The flattened cell columns, 130-160μ (or 14-18 cells) in thickness, form cones each with 2 or 3 rows of cells diverging out toward the base of the cone which, in a majority of cases, faces the diaphyseal side. The matrix between them increases toward the base. The vesicularized cells, often absent in the first region, are usually present here and 32-50μ (or 1-3 cells) thick. (3). "Perioststreifen" region is the region where the anastomosing branches of blood vessels from the articular cartilage pierce the cartilaginous plate. Here cone formation is more abundant. The cones are smaller and cell columns usually contain no or few vesicularized cells. (4). The marginal region is the outermost margin of the plate, normally thinner and less active than the inner region but here relatively thicker and containing a few regular cell columns pointing outward. The primary trabeculae in the second region are short (80-250μ), shorter than the abundant horizontal branches (160-320μ). In many places, especially the third region, the epiphyseal plate is sealed on both sides by horizontal bony plates, which makes further ossification difficult, if not impossible. The direction of the

trabeculae in this region is parallel to the surface of the epiphyseal plate, in the direction of the enlarging metaphysis, and perpendicular to the longitudinal axis of the shaft. The growth of the metaphysis on this account will be very slow and sidewise as well as forward. The primary marrow cavity is small. Erosion, if present, would probably be very slow and irregular. In places where no vesicular cells are present, the blood vessels erode through the flattened cell columns or the matrix. The cartilaginous bar in the cancellous bone is scanty. If present, it may contain flattened and proliferating chondrocytes. The trabeculae have longer horizontal branches and form larger marrow cavities. Osteoblasts are fewer in number and mostly flattened in shape. Osteoclasts are fairly common. Periosteal ossification is extensive and there are many subperiosteal buds. The periosteal trabeculae are thin but densely interlaced, extending to the epiphyseal plate with 3-9 layers (80-130μ). The ossification in the articular cartilage is in general more active than at the epiphyseal plate.

Cone formation in the second and third regions is a striking feature (Fig. 9, 10, 13). Each is always confined by the anastomosing branches. Some of them may have the base facing the epiphyseal end, but mostly the base is on the diaphyseal side. The form and size of these conical, triangular, or wedge-shaped clumps depend upon how the blood vessel branches. They wedge into the proliferating zone, have sharp boundaries and divide the latter into isolated areas. The matrix in the cone is more abundant than the cellular elements and takes a deep purple color in hematoxylin-eosin preparations, in contrast to the pinkish color in the matrix of the proliferating zone. In azan stain the former takes a light greenish or bluish tint and the latter a deep blue. The flattened cell columns are irregular and the hypertrophied cells, if present, are usually not in column formation but scattered here and there inside of the cone. Most of the cells are of the intermediate type: enlarged but not vesicularized; they may be round or sickle-, spindle-, triangular- or oval shaped. Proliferating cells are rare in these cones. Pyknotic cells may be present but are never confined to any area of the plate. Near the diaphyseal surface of the plate phagocytic cells may precede the vascular erosion.

3. *Severe degree of hypoplasia of the distal epiphysis of radius* (Fig. 6, 8). The epiphyseal plate is more extensively perforated and disintegrated and the position of the remaining plate more oblique.

The perforating canal occupies most of the metaphyseo-epiphyseal junction. The arrangement of the cones is extremely irregular. Microscopic structure is similar in nature to that described in the intermediate cases. Even the short stumps of the epiphyseal plate remaining in the distal end of the tibia are similar to those in the intermediate cases.

4. *Epiphyseal plate in the metacarpus* (Fig. 7, 12). Since the cannon bone of sheep originates from an incomplete fusion of the third and fourth metacarpi or metatarsi, two separate epiphyseal plates joined together by the cartilaginous roof of the sagittal notch are retained. In the anterior cannon bone of newborn Ancons, both plates are atrophic, in different degree of disintegration, and each possess a large perforated canal; that of the lateral side is more extensive and more proximally located than the medial one. The perforated canals are always eccentric. The histological structure of the epiphyseal plates and of the trabeculae reveals the same situation as in the distal epiphyseal plate of the radius. However, here the horizontal bony plate seals the third region of the plate more frequently. The proliferation and growth of the chondrocytes in the fourth region, however, are more interesting. The cell columns extend horizontally to the perichondral side and then turn 135-180° over the blocking bony plate to the diaphyseal surface pointing toward the center of the bone. Ossification here is sidewise and the metaphysis thereby is further enlarged. The denseness of the cancellous bone varies with the activity of the epiphyseal plate. It is thin and loose when the endochondral ossification is inactive and vice versa. Therefore the medial side of the metaphysis often has thicker and denser bony spicules but a thinner periosteal collar than that of the lateral.

The articular cartilages of the normal and Ancon bones show differences in activity which are similar to those of the epiphyseal plates but less extreme. As a consequence the trabecular meshwork in the epiphyseal marrow cavity also varies in density. The more active articular cartilage forms a thicker and denser meshwork than does the less active one (compare Fig. 3, 4, with 5 and medial and lateral sides of Fig. 7). Formation of conical clumps can also be found in the articular cartilage, but they are not as striking. The effect of the blood vessel on cartilaginous structure is also the same.

c. *Ground sections of the shaft of metacarpi.* Transverse sections of the middle of adult metacarpal shafts were ground, toned with silver nitrate to bring out the boundaries of the Haversian systems, and mounted in dry balsam. It was found that the Haversian systems in the adult metacarpus of both breeds were grouped into different patches. The arrangement, size, and number of Haversian systems in these patches vary one from another. One patch may have the greatest diameter of the Haversian systems all parallel to the circumference of the bone while others may be perpendicular or oblique. The least and greatest diameters of the Haversian systems in the two kinds of animals are, on the average, very similar (Ancon: 121-158μ, normal: 130-156μ). The number of complete Haversian systems in a square area of 857μ is, on the average, 30 in the normal and 20 in the Ancon sheep. The Haversian systems are more regular and compact in the normal bone (Fig. 14) than in the Ancon (Fig. 15). It seems clear that the normal compact bone of the metacarpus is stronger and more solid than that of the Ancon.

Discussion and Conclusion

The present study indicates that the onset of the Ancon condition occurs prior to birth and that a study of the embryology may be instructive. As shown by the article on the skeletal growth of Ancon sheep, distal segments are more retarded than proximal ones and the hindleg more than the foreleg. It seems that the rate of retardation is closely related to the degree of hypoplasia in the epiphyseal plate and to its position, and to a much lesser extent also to the activity of the articular cartilage. Using thickness, length and obliqueness of the remnant and size of the perforating canal as criteria it appears that the degree of epiphyseal hypoplasia, in descending order, occurs in the following sequence: distal radius, distal metacarpus, and proximal radius in the foreleg; distal metatarsus, distal tibia, proximal tibia and distal femur in the hindleg.

According to Bergmann (5) and Payton (39) in man and the pig most of the increase in length of humerus and radius-ulna is contributed by the epiphyses away from the elbow whereas increase in length of femur and tibia-fibula occurs chiefly at the epiphyses near the knee joint. The situation in sheep confirms their observations if the early fusion of the epiphyses of distal humerus, proximal radius, proximal

femur and distal tibia, as reported by Hammond and Appleton (18), can be considered proof. In the Ancon material the epiphyses of the humerus are fairly normal but the distal epiphysis of the femur is often severely perforated. This probably accounts for its being more retarded than the humerus. The situation in the radius and tibia can be explained in like manner. The cannon bone has only a distal epiphysis and this is disintegrating in the metacarpus and has almost completely disappeared in the metatarsus. The latter, therefore, is more reduced than the former. The gradient of increasing proximo-distal retardation can be explained on the same basis.

The curvature of the femur of Ancon sheep does not show any difference from the normal in the newborn but becomes distinct, in some cases, as a result of further growth. The difference in condyloid angle sometimes is barely visible in the newborn but is more striking in the older animals. The eccentric disintegration on the posterior side of the distal plate of the femur suggests that the curvature of the femur as well as the condyloid angle are the results of more active growth in the anterior than in the posterior part. The late appearance of this distortion probably is due to the late development of the femur and there may also be a late onset of disintegration in the epiphyseal plate.

In bent femurs there are radiating trabeculae along the thickened posterior wall of the shaft (Fig. 1 K). These are relatively few, short and not striking when compared with those found in the tibia of chondrodystrophic chicken embryos (Landauer, 24). These trabeculae decrease with age. In the adult there is only a slight indication of their presence.

Microscopic examination of bones from our newborn Ancon lambs strongly suggests that the metaphyseal enlargement is at least partially due to the obliqueness of the epiphyseal plate. Since the central region is more seriously fragmented and perforated, it is less active and lags behind, whereas the relatively more active peripheral part is able to shift forward. Endochondral ossification, whenever possible, will occur obliquely outward to enlarge the metaphysis. Furthermore, the marginal cell columns sometimes extend horizontally outward to avoid the blockage by the bony plate, and this also tends to increase the diameter of the metaphysis. The presence of a strongly demarked zone enlargement in the metaphyseo-epiphyseal junction is an indication of such activity. Weinmann and Sicher (49), attributed the

peculiar mushroom shape of chondrodystrophic bone, to a "consequence of the dual growth mechanism of articular cartilage." We have no direct evidence to support this point. However the articular cartilage is less affected than the epiphyseal plate. Its appositional increase, therefore, in comparison to length increase by the plate, presumably is relatively greater and this probably is responsible for the simultaneous enlargement of the epiphysis.

In the newborn Ancon the shafts of the long bones, with the exception of the humerus, are thicker than normal. Microscopically the side with the more severe perforation of its epiphyseal cartilage has thicker periosteal bone in the metaphysis. These two kinds of ossification seem to be antagonistic. Cowdry (12) described chondrodystrophy as a situation in which "cartilage cells in the epiphyseal plates of their long bones go on strike but periosteal bone continues to be laid down on the shaft." The sheep periosteal bone formation not only continues but seems to be intensified. Murray (35) explained the increase in thickness on the concave side of the bent bones by the presence of greater subperiosteal space resulting from loosening of the periosteum and bending of bone. His suggestion may explain the thickening of the bone shaft in the newborn Ancon as a result of metaphyseal enlargement. In the adult Ancon the shaft is thinner than normal, especially in the hindleg. Even the concave side of the most extremely curved femur in our collection is thinner than normal. Murray's suggestion cannot be applied here.

The dome-shaped permanent architecture of the humerus is already established and the architecture of the femur is just beginning to be laid down in the newborn sheep. A correlation between the establishment of the permanent system and walking, as suggested by Townsley (47) is unlikely to exist in our cases. In considering the functional relationship, one would expect to have a modified trabecular pattern in the adult humeri since most of the Ancon forelegs are crippled. Our adult humeri failed to show such modification. On the other hand we have found that the curvature and position of the epiphyseal plate, the direction of the blood vessels and the pattern of the trabeculae are interrelated. Nunnemacher (37) has already come to the conclusion that the blood supply is closely related to the activity of epiphyseal plate. With Harris (19) and his collaborator (20) one may conclude that the arrangement of the trabecular system is determined by hereditary agencies. These factors influence the activity of the cartilaginous

plate of the epiphysis, determine the direction of the trabeculae and therefore the permanent architecture.

Histological sections of sheep bones reveal the presence of proliferating blood vessels in normal epiphyses as well as in those of Ancon sheep. These are derived from vascularization of the epiphyseal marrow cavity and from that of the articular cartilage. The blood vessels in the latter situation are similar to the "Perioststreifen" in human chondrodystrophy. The perforation in Ancon sheep is much more extensive and the canals form a network instead of passing straight through. This network isolates the cell column zone of the plate into conical clumps of various sizes and shapes. The anastomosing vessels further block the linear growth of the cartilage and thereby intensify the irregular arrangement of cell columns. As a result the latter are arranged obliquely and are conical in longitudinal sections. As the perforating channels gradually increase in size and bony plates begin to deposit on the surface of the cartilage, the plate is fragmented into isolated patches. These islands lose their supply of new chondrocytes, and become further fragmented and resorbed.

Except for the abnormalities found in the occipital region, the skull appears to be normal. The scolioses and the lordoses of the lumbar vertebrae and the enlargement (flaring) of the metaphyses of some leg bones are the same in the Ancon sheep as in human chondrodystrophy.

The histological structure of the epiphyseal cartilage of Ancon sheep is also similar to modifications caused by other physiological changes. The most striking resemblance to the epiphyseal plate of the newborn Ancon is found in skeletons of ageing mice and rats. Silberberg and his collaborators (42, 43) have found very similar conical structures and similar staining properties in control mice and in animals which had been subjected to thyroxin, sex hormone, underfeeding and breeding influences. The disintegrating epiphyseal plates of the Ancon are similar to those of the control and thyroxin injected rats used in Evan's laboratory (3, 4, 36). Dawson (14, 15) observed the conical structures in the cartilaginous plate of rat bones during epiphyseal union. In addition to the above mentioned cases hypoplasia and conical formations in the epiphyseal plate were found in cases of riboflavin, pantothenic acid, pyridoxine deficiencies in mice (29, 30, 44) and other types of malnutrition (rat, 40; chicken, 26, 27). On the other hand, the epiphyseal plate in sheep chondrodystrophy differs from these experi-

mental cases and those involving ageing in that our material consists of newborn animals, by the fact that the cancellous trabeculae are altered differently and, most important of all, in regard to the intensified periosteal ossification.

As far as the microscopic structure of the cartilage is concerned, hypoplasia, conical formations and fragmentation probably can be caused by many agents, hereditary as well as environmental. The hypoplastic epiphyseal plate alone may not be typical of chondrodystrophy by itself, but it is a characteristic part of the syndrome when the age and the related structure of the cancellous bone and the periosteal collar are considered together.

The deep staining matrix in the conical clumps is very similar to what is usually described by investigators as calcified matrix. McLean and Bloom (32) investigated calcification in normally growing bone and found salt deposition mainly in the longitudinal matrix, only 3-4 cells ahead of the advancing marrow, and confined to the hypertrophied cell area. In the hypoplastic cartilage of our sheep and probably also in ageing animals, when good staining is obtained, the whole area of the conical clumps takes a homogeneous purple or blue tint in hematoxylin-eosin stain. These sections were decalcified. How much calcium was left to take the basic hematoxylin is a question. These cones are probably too large to be considered calcified areas. Harris and his collaborator (19, 20), from a study of aborted chondrodystrophic fetuses and of a Dexter calf, were the first to consider mucoid degeneration as the essential feature of chondrodystropy. Since their material was more degenerate and younger it cannot, properly, be compared with our observations on sheep. Sections of the normal and Ancon radius (newborn, formalin fixation) were stained in 0.2% aqueous solution of toluidine blue (National Aniline and Chemical Co., C. I. No. 925). The matrix stained red violet, typical for chondromucin, but it failed to show any color difference between the epiphyseal plates of the normal and of the proliferating zone and conical area of the Ancon sheep. In order to find out whether the conical clumps in Ancon sheep represent mucoid degeneration more specific preparations are needed.

The blood vessels and their adjacent chondrocytes and matrix have the same relationship in human developmental abnormalities, as shown by Bonola's (7) Fig. 4 and Burckhardt's (9) Fig. 10. In Wilton's (50) normal fetus (Fig. 20) the cartilage is also penetrated by blood vessels which form the "Knorpel Canäle" and which have the same relation

to the surrounding structures. It seems that the coarser fibers, more deeply stained matrix and smaller size of the chondrocytes around the blood vessels in the cartilage are normal phenomena not the characteristics of any pathological condition. However, the pathology results when there is an increase in number of these structures and their presence intensifies the hypoplasia of the cartilage.

The structure of the epiphyseal plate in Ancon sheep varies from the normal condition to one of nearly complete disintegration depending upon which part of a particular bone, which skeletal segment or which animal is used for study. The literature on human chondrodystrophy indicates that the same variability exists in human material. At any rate, Snoke (45) has concluded that "in the same individual the several groups of bones may vary in the severity of their involvement."

Von Hassen (48) and Andreeva (1) found more Haversian canals in the compact bone of the metacarpus of wild races than in that of the domestic ones. Our findings indicate fewer and more irregular Haversian systems in the Ancon metacarpus than in the normal bone. Since the structure of adult bones are influenced by the activity of the animal, it cannot be decided whether the aforesaid differences in the metacarpi of normal and Ancon sheep are inherited or secondary effects.

Considering the evidence as a whole one may conclude that the dwarfism of Ancon sheep represents a mild form of chondrodystrophia hypoplastica.

Summary

1. Ancon sheep are disproportionate dwarfs which transmit this condition as a recessive character.

2. The skeletons of 14 heterozygous (normal) and 50 homozygous (Ancon) sheep were used for this study.

3. A general survey of the Ancon skeleton shows that some animals have abnormalities in the occipital region of the skull, scoliosis and lordosis of the vertebral column, greater curvature of the shaft and acute angle of the condyles of the femur, and enlargement of metaphyses and exostosis-like formation of the more affected leg bones. Sesamoid bone formation may also occur in the crippled joints.

4. Hemisections of the long bones indicate that the epiphyseal structure varies with region, bone and individual. In newborn Ancon sheep the epiphyses of the humerus and the proximal ends of the femur and

radius are nearly normal. Those of the distal ends of radius, metacarpus and femur, and of the proximal end of the tibia are in a process of disintegration which is characterized by eccentric perforating canals in which long and straight trabeculae of membraneous origin are found. The distal epiphyses of the tibia and metatarsus have practically disappeared.

5. The differences in the rate of retardation between the proximal and distal segments on the one hand and the fore- and hindlegs on the other are explained.

6. Cross sections of the middle of the shafts of long bones indicate that these bones are thicker in Ancon than in normal lambs, but that they are thinner in adult Ancon sheep. Ground sections of the compact bone of the metacarpus of adult sheep indicate that the Haversian systems in the Ancon bone are more irregular in arrangement and form and numerically fewer in a unit area.

7. The permanent trabecular architecture of the long bones of sheep is considered to depend primarily on the curvature and activity of the epiphyseal plate.

8. Causes of the metaphyseal enlargement and femur curvature were discussed.

9. The microscopic structure of the hypoplastic cartilage of the newborn Ancon sheep has considerable similarity to normal ageing skeletons and, to a lesser extent, to some types of experimentally induced abnormalities, but differs from all of these in that these structures are present at birth, are more numerous and by showing increased periosteal ossification and other trabecular changes.

10. The Ancon mutation of sheep produces a mild type of chondrodystrophia hypoplastica.

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CRIPPLING IN CHONDRODYSTROPHIC (ANCON) SHEEP

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A recessive mutation leading to disproportionate dwarfism, occurred towards the end of the eighteenth century in a flock of sheep belonging to Seth Wright of Dover, Massachusetts. In 1919 another mutation for shortleggedness appeared among sheep of eastern Norway (4). These two independent mutations have very similar manifestations, the details of which will be described elsewhere. Both kinds of sheep were shortlegged and crippled. It was the crippling that led Shattuck (3) to give the name "Ancon" to these sheep. Ancon is a Greek word which signifies elbow. Ancon sheep became common in New England for a time because of their inability to jump over stone fences and to damage crops. However, the lambs were less able to stand up than others for sucking without assistance and it was found difficult to drive these crippled animals to market. For these and other reasons the American Ancons eventually became extinct. The last flock known consisted of eight animals in Rhode Island in 1876. As the Ancon sheep have enlarged joints, an increased diameter and a decreased length of the long bones, they have been diagnosed as chondrodystrophic. Our macro- and microscopic study of the skeleton of these animals led to the same conclusion. Occasionally, the two condyles of the distal end of the cannon bone, particularly the metacarpus, were found to be irregular and the medial and lateral phalanges to be unequal in length. Pierre Marie's description of "main en trident" as one of the characteristic symptoms of human chondrodystrophy and Bertolotti's (2) observation of disproportionate shortening of the metapodia seem to be phenomena similar to our findings in the chondrodystrophy of Ancon sheep. The following report will analyse the structural deviations in relation to crippling of Ancon sheep.

Material and Methods. The material used for this analysis came from the descendants of the Norwegian Ancon breed. We had 50 preserved skeletons and a flock of 13 Ancon sheep ranging in age from

newborn to adult. Most of the preserved specimens had complete phalanges and intact hoofs. Analysis can easily be made by judging the structure of the cannon bones, phalanges and especially the location and wear of the hoofs. Roentgenographic study of this region in three living lambs, observed at monthly intervals from birth to 3 months of age, was of great help. The types of crippling observed in preserved specimens were verified from the living flock of the Department.

Observations (Fig. 1, A-M). Ancon lambs when newborn, often cannot stand up; but they all begin to walk after a few days of nursing. Most of these weak lambs begin to show crippled behavior without any evident gross morphological abnormality. After the second or, more often, the third month of postnatal life various degrees of bending of the joints and differences in the angle of slanting of the toes begin to appear. In cases of severe crippling of adults it is obvious that the cannon bone presses down toward the ground and that the angle of the metacarpo- or metatarso-phalangeal joint deviates from normal by as much as 90°. In the most abnormal cases the cannon bone touches the ground and the digits turn over to one side, most frequently anterio-laterally. The lightly crippled leg is more difficult to detect, unless the animal stands still. Serious deformity occurs chiefly in the foreleg. When the hindleg is crippled the condition tends to be less extreme.

The crippled legs are arbitrarily divided into three types and a brief description of each type will here be given.

1. *Mild type* (D-J). The digit or digits, instead of being parallel to each other, are slanted due to bends at the metacarpo- or metatarso-phalangeal, the interphalangeal, or both joints. In these joints, the capsule is thickened and more fibrous, the ligaments are strengthened, the metaphyseal ends are enlarged, and the bone surface is roughened. The position of the digits appears normal when the leg is not under pressure.

2. *Intermediate type* (K). The metacarpo-phalangeal joint forms an angle of about 135°. Most of the articular surface of the cannon bone and the phalanges are deprived of hyaline cartilage and instead have a tight fibrous and roughened, uneven surface; the capsule is always tough and may be partially ossified into a bony plate or plates, at one or both sides of the metacarpo-phalangeal joint. One side of the metacarpal condyles may have an exostosis-like enlarge-

ment with smooth articular surface blocking the upward shifting toes. The distal end of the metacarpus is enlarged and has a rough surface. The proximal ends of the phalanges are similarly enlarged and spiny. The affected joints are fibrous and lose their free mobility. The medial surface of the medial hoof faces the ground and is much thickened. The hoofs become long and curved because there is no wear.

3. *Extreme type* (L-M). The metacarpus is pressed down to the ground and the digits are shifted to their anterio-lateral surface. The metaphyses of the metacarpus and the phalanges are enlarged and spiny. The metacarpo-phalangeal joint forms an angle of 90°. The articular surfaces are completely fibrous with little mobility. The ligaments and capsules are thickened and massive. The medial and lateral phalanges are bound together at their proximal ends by fibrous tissue. The medial surface of the phalanges faces the ground. The medial surface of the medial hoof faces the ground and is thickened. The hoofs are long and curved due to lack of wear.

Figures A to M are diagrammatic representations of the types of crippling shown by our preserved specimens and further verified in the living flock. These diagrams illustrate the following facts: (1) the abnormal bending occurs either (most commonly) at the metacarpo- or the metatarso-phalangeal joint (H-M), or at the interphalangeal joint (D-E), or at both (F-G). In fact any effect on the interphalangeal joint influences always at the same time the adjacent metcarpo- or metatarso-phalangeal joint. (2) The bending may be in only one of the digits (medial, H; lateral, F-G) or in both (E, H-J). (3) The bend may be inward toward the medial line (D, E, G) or outward, either toward the lateral side (F, I, K-M), medial side (J), or both (H). (4) When both toes are affected the bending may be opposite in direction (E, H) or in the same direction either both mediad (J) or both laterad (I, K-M). (5) The digits may also slant more than usual toward the anterior side.

Since unequal length of the digits is more frequent in the forelegs, which bear more body weight, and is associated with the more severe crippling, the unequal length of digits was first suspected to be the cause of crippling. It was thought that perhaps the single longer toe was unable to support the body weight without the help of the other toe. This idea seemed to be further supported by the discovery of some specimens with unequal phalangeal bones but in which the leg

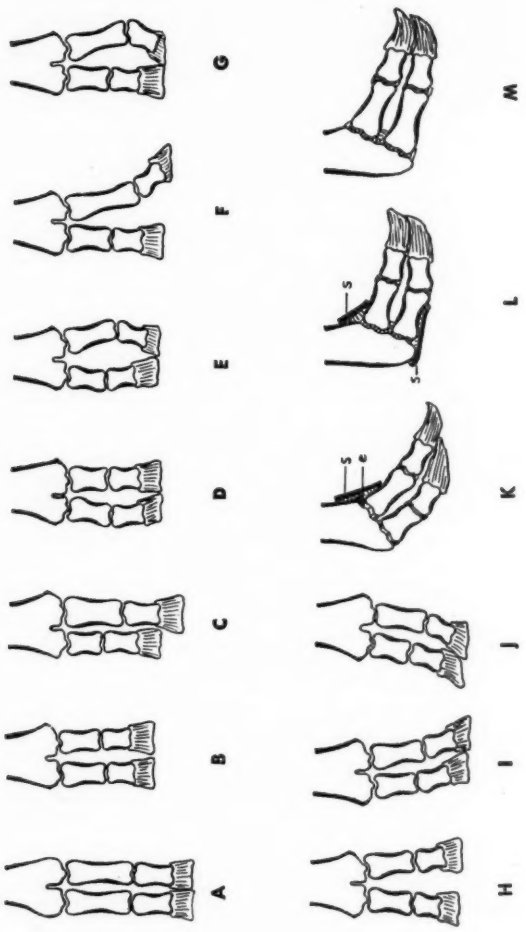

FIGURE 1.

Diagrams showing types of crippling in Ancon sheep. Left side in each diagram is the medial side of the leg, and the right side the lateral. In drawing the diagrams the diameters of all first phalanges were set at 5 mm. and of the second phalanges at 4 mm., the length were calculated in proportion. The unequal shortening of the phalanges in most cases was, therefore, exaggerated.

e — exostosis-like eminence; s — sesamoid bone

A. Normal, A44, 11¼-year male; right foreleg; metacarpal length, medial 155.8 mm., lateral 155.5 mm.; first phalangeal length, medial 35.5 mm., lateral 36.5 mm.; second phalangeal length, medial 24.5 mm., lateral 25 mm.
Normal leg

B. Ancon B4 1 1/6-year male; right foreleg; metacarpal length, medial 111 mm., lateral 108 mm.; first phalangeal length, medial 39 mm., lateral 38 mm.; second phalangeal length, medial 21 mm., lateral 25 mm.
Not crippled.

C. Ancon A11, 4-year male; left foreleg; metacarpal length, medial 67 mm., lateral 65 mm.; first phalangeal length, medial 28.4 mm., lateral 34.7 mm.; second phalangeal length, medial 20.3 mm., lateral 19.6 mm.
Not crippled.

D. Ancon A2, 10-year female; right foreleg; metacarpal length, medial 71 mm., lateral 71 mm., first phalangeal length, medial 27 mm., lateral 23.5 mm.; second phalangeal length, medial 18.7 mm., lateral 19 mm.
Interphalangeal joint of medial digit bent toward medial side.

E. Ancon B2, 4-year male; right foreleg; metacarpal length, medial 69 mm., lateral 74 mm.; first phalangeal length, medial 29 mm., lateral 37 mm.; second phalangeal length, medial 23 mm., lateral 25 mm.
Interphalangeal joints away from median line.

F. Ancon A10, 5 1/3-year female; right foreleg; metacarpal length, medial 63.4 mm., lateral 63.4 mm.; first phalangeal length, medial 27 mm., lateral 39 mm.; second phalangeal length, medial 20 mm., lateral 26 mm.
Lateral digit slanted laterad.

G. Ancon B15, 3½-month male; right hindleg; metatarsal length, medial 80 mm., lateral 80.2 mm.; first phalangeal length, medial 28.4 mm., lateral 38.5 mm.; second phalangeal length, medial 19.3 mm., lateral 21.2 mm.
Lateral interphalangeal joint away from median line.

H. Ancon A8, 6-year female; left hindleg; metatarsal length, medial 67 mm., lateral 67.2 mm.; first phalangeal length, medial 26.8 mm., lateral 27 mm.; second phalangeal length, medial 17 mm., lateral 18 mm.
Each digit forked out, away from median line.

I. Ancon B14, 3½-year male, left hindleg; metatarsal length, medial 73.4 mm.; lateral 72 mm.; first phalangeal length, medial 28.7 mm., lateral 27.6 mm.; second phalangeal length, medial 20 mm., lateral 20 mm.
Both digits slanted toward the lateral side.

J. Ancon A11, 4-year male, right foreleg; metacarpal length, medial 67 mm., lateral 67.2 mm.; first phalangeal length, medial 28 mm., lateral 35 mm.; second phalangeal length, medial 21 mm., lateral 20 mm.
Both digits slanted toward medial side.

K. Ancon B2, 4-year male, left foreleg; metacarpal length, medial 84 mm., lateral 67 mm.; first phalangeal length, medial 28.7 mm., lateral 27.6 mm.; second phalangeal length, medial 23 mm., lateral 35 mm.
Digits slanted to lateral side, metacarpo-phalangeal joint bent to form 135°.

L. Ancon A38, 8-year female, right foreleg; metacarpal length, medial 82 mm., lateral 64.5 mm.; first phalangeal length, medial 34 mm., lateral 32 mm.; second phalangeal length, medial 23 mm., lateral 25 mm.
Digits slanted to anterio-lateral side, metacarpo-phalangeal joint bent to form 90°, metacarpus pressed down to ground.

M. Ancon B4, 1 1/6-year male, left foreleg; metacarpal length, medial 95 mm., lateral 72 mm.; first phalangeal length, medial 38 mm., lateral 31 mm.; second phalangeal length, medial 20 mm., lateral 23 mm.
Digits slanted to anterio-lateral side, metacarpo-phalangeal joint bent to form 90°, metacarpus pressed down to ground.

was not crippled and the total length of the digits was identical, due to a balancing by alternating arrangement, of long and short bones (B). The length of the hoof can always compensate for slight differences in length. However, upon careful analysis the differences in digital length were found not to be the primary factor of crippling, though they may intensify the phenomenon. The longer toe alone is able to support the weight without crippling (C). On the other hand, cases with the two digits nearly equal may be only slightly bent (D) or distorted to such an extent that the animal actually walks on the distal end of the metacarpus instead of on its toes (L). Furthermore the bending may be either toward the longer (J, K) or the shorter (I, M) side, or each slanting its own way (E, G).

The unequal size of the metacarpal condyles was then suspected to be the cause of crippling. As will be described elsewhere, the sagittal notch of the cannon bone often is irregular in form, even in the newborn and other young stages, and the two condyles often are not on the same level. Our data show that the lateral side is, on the average, shorter, due to a higher level of the lateral condyle. The frequency of shortening is greater on the lateral than the medial side and greater in the foreleg than the hindleg. This seems to be correlated more with the occurrence of crippling; the bending being more frequently toward the lateral than toward the medial side and more severe in the foreleg than in the hindleg. These facts provide no solution of the problem because bent toes occur in legs with practically equal length on the two sides (D, F, G, H, J), and because the degree of bending at times is not in accord with the difference in length. Moreover, one cannot explain in this way the origin of forceps-like (E), forked (H), inward (D, G) or outward (F) distortions.

The blocking eminence (K, e) and extra sesamoid plates (K, L, s), described previously, furnished the suggestion that the mechanism of crippling may be related to the supporting strength of the joints. However, although adult material supplies different types of crippling, it is difficult to decide what may be cause and effect. Our observations are, therefore, mainly based upon newborn specimens and the X-ray films taken from living lambs. From these data it was finally possible to attribute the mechanism of crippling to the obliqueness of the articular surface. This will explain the types of crippling found in our material as follows:

1. Articular surfaces of both sides normal, digits equal, the joints are normal (A).

2. Articular surfaces of both sides normal, digits compensated to equal length, the joints are normal (B).

3. Articular surface of one side normal, the digit of this side long, the joints are normal and the single long digit is able to support the weight (C).

4. Articular surfaces of one side normal, other side slightly oblique, digit of the normal side short, the joint of the oblique side with long digit is slightly bent (D, F, G).

5. Both articular surfaces slightly oblique and facing outward, digits unequal in length, the joints are bent with digits forked away from the median line and the longer side bent more severely (E, H).

6. Both articular surfaces slightly oblique, one facing inward, the other outward, digits are slanted in one direction (I, J).

7. Both articular surfaces very oblique, one side facing inward, the other outward, the outward side is blocked by bony eminence or extra sesamoid bone, both digits are slanted in the same direction (K).

8. Both articular surfaces very oblique, one facing inward, the other outward, no blocking structure or an ineffective one, both digits are slanted in the same direction and the metacarpus is pressed down to the ground (L, M).

9. Interphalangeal articular surface oblique, facing outward, second phalanx is slanted outward (F, I, J, etc.).

10. Interphalangeal articular surface oblique, facing inward, second phalanx is slanted inward (D, E, G).

11. Anterior surface of condyles cannot support toes, the toes are bent anteriorly.

From the above explanation of crippling, one might expect cases in which the metacarpo-phalangeal joints form angles of 90°, the medial digit slanting to the medial side and the lateral one to the lateral side to form the extreme type of (H). Such cases failed to occur in our material.

Discussion. The above analysis shows that it is neither the difference in level of the two condyles of the cannon bone nor the difference in length of the digits, but the obliqueness of the articular surface that causes the crippling of chondrodystrophic sheep. Our

microscopic findings showed that the articular cartilage and particularly the epiphyseal plate of the cannon bone are hypoplastic. When the medial and lateral epiphyseal plates were compared, the lateral side often was found to be more hypoplastic and more severely perforated. Furthermore, the perforating canal often was eccentric. The obliqueness of the articular surfaces is, therefore, the result of the difference in activity of the cartilage, mainly in the epiphyseal plate, but partly also in the articular cartilage. Since the frequency of occurrence of an irregular articular surface is greater on the lateral side of the cannon bone than on the medial and more common in the foreleg than in the hindleg, there are more cases of lateral bending of the digits in the foreleg. As the level of the condyles, the form of the sagittal notch, as well as the obliqueness of the articular surfaces are all due to differences in the activity of the cartilage, there will be a close relation, but the former two conditions are not the cause of the latter.

Weakness of the joint already exists in the newborn, but the weight of the body at this stage is not great enough to press down the bones and to bend the joints. As a consequence although such lambs have difficulties in standing up and show crippling behavior, they have no externally detectable distortions of the joints. The defects find visible expression as the body weight increases. The cause of crippling, therefore, is congenital, but the thickening of the capsule, the fibrous and slanted joints, and other related structural changes are secondary results of the crippled condition.

In human chondrodystrophy Pierre Marie's "main en trident" is a similar symptom. Bertolotti (2), by using radiographic measurements of the hands and feet of a chondrodystrophic family, showed irregularity in length of the metapodia. Balme and Reid (1) demonstrated the "main en trident" by skiagram and stated that "the divergence of the middle and ring fingers is seen to be due to excessive expansion of the heads of their proximal phalanges." They further showed "the inequality in the length of the metatarsals and the distortion of the terminal phalanges" in the foot. There is a strong suggestion that these cases of "main en trident" and metapodial micromelia may be due to irregular cartilage activity, and the same may hold for bending of knee and wrist joints as it frequently occurs in human chondrodystrophy. The effect in human beings is not as serious and does not cause crippling because of functional differences.

Summary. An analysis of the crippling in Ancon sheep, based upon 50 preserved skeletons and a flock of 13 living Ancons, and Roentgenographic study of three living lambs from birth to three months of age, showed that crippling in Ancon sheep is neither due to the presence of different levels of the condyles of the cannon bone nor to unequal length of the medial and lateral digits but that it is caused by the obliqueness of the articular surfaces. This obliqueness is due to differences in activity of the cartilage. The mode of origin of different types of crippling was illustrated in diagrams.

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THE EFFECT OF BIOTIN ON MITOTIC ACTIVITY IN THE LIVER OF THE MOUSE[1]

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INTRODUCTION

In a previous paper we have reported that mitotic activity may be stimulated in the mouse liver by the intraperitoneal injection of pulped liver, kidney, or egg yolk (Wilson and Leduc, 1947). Boiled or autolysed liver was as effective as fresh liver. Liver, kidney and egg yolk are all high in biotin, and biotin is thermostable. Furthermore, we had used the autolysed liver in the expectation that by liberation of some active agent the effect could be speeded up, but this did not occur; and biotin is not freed from combination in the liver by autolysis (György, *et al.*, 1939). All these facts pointed to the desirability of investigating the effect of biotin on the mitotic activity of the liver.

Our experience in producing biotin deficiency in mice has already been reported (Wilson, Leduc, and Winston, 1949). The present paper is a report of experiments performed to study the mitotic activity in the livers of biotin deficient mice, of mice which are being cured of the deficiency and of non-deficient mice that are being treated with biotin. Our results indicate that biotin is not the factor responsible for the stimulation of mitosis in the liver by the injection of pulped liver, kidney or egg yolk.

MATERIAL AND METHODS

The mice used for these experiments were from our own colony in which several inbred strains are being maintained. They were mostly from new albino strains that are being selected particularly for vigor of growth and reproduction. They are kept in a room with

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the temperature controlled at 75°-78° F. and fed Purina laboratory chow.

For the production of the biotin deficiency, glass cages with raised floors of wire mesh were used. The diets have been described and discussed in detail elsewhere (Wilson, Leduc, and Winston, 1949). To avoid any secondary problems that might arise from the use of sulfaguanidine, we have not employed diets containing this drug although we have found them to be the most effective in producing the deficiency. The diet in general use is as follows: G.B.I. vitamin-test casein, 10 per cent; egg white,[3] 20 per cent; Argo corn starch, 29 per cent; Crisco, 20 per cent; Mazola corn oil, 4 per cent; Squibb's cod liver oil, 2 per cent; G.B.I. salt mixture USP XII, #2, 7 per cent; G.B.I. powdered brewer's yeast, 8 per cent. In most experiments the mice are weaned onto this diet when 16 to 19 days of age and the majority of them show the usual signs of deficiency in 4 to 5 weeks. The fact that in some of the animals the deficiency symptoms never develop introduces an element of uncertainty in those experiments where changes in the liver during the development of the deficiency were studied.

The deficiency was cured by the subcutaneous injection of biotin[3] and by the substitution of a basal diet for the diet containing egg white. To determine the changes in mitosis in the livers of the animals so treated, one mouse was killed each day during the course of the experiment and its liver prepared for microscopic examination.

For the determination of the mitotic activity of the liver, the method of Wilson and Leduc (1947) was used. The mice were killed by a blow on the head, decapitated and bled thoroughly. A piece of the large left lateral lobe of the liver was fixed in Bouin's fluid, sectioned in paraffin at 5μ, and stained in Delafield's hematoxylin and eosin. All the mitotic figures in a single section were counted under an oil immersion objective and this number was reduced to a standard area and nuclear density by the following formula:

$$\text{mitotic activity} = \frac{\text{number of mitotic figures}}{\text{area of section} \times \text{nuclear density}}$$

The nuclear density of the section was established by counting all

[3]We are indebted to Henningsen Lamesa, Inc., Lamesa, Texas, for the powdered egg white and to Merck and Company, Inc., Rahway, N. J., for the crystalline biotin.

the nuclei in a standard area, and the area of a standard projection of the section was measured with a planimeter.

Experimental

Two experiments were performed to observe the changes in mitotic activity in the livers of young mice during the development of biotin deficiency. Sixteen-day-old mice were placed on an egg white containing diet and on the corresponding basal diet and they were subsequently killed at intervals through 30 days to determine mitotic activity. No difference between the control and experimental groups was found to which we could assign any significance.

We have treated deficient mice in two ways in order to study the effect of biotin on the mitotic activity of the liver: (1) by substituting basal diet for the egg white containing diet, and (2) by the subcutaneous injection or biotin.

Control diets have been substituted for diets containing egg white in 4 experiments. In experiment 150B (Fig. 1) the mice were placed on an egg white containing diet at 16 days of age and showed mild deficiency symptoms after 20 days. After 29 days on the egg white diet, when deficiency symptoms were definite, they were transferred

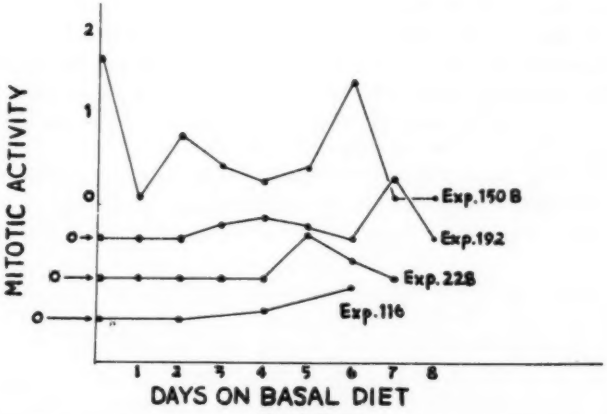

FIGURE 1

Mitotic activity in the livers of biotin deficient mice after a basal diet was substituted for the diet containing egg white. Each point on the curves represents the mitotic activity in the liver of a single animal.

to the basal diet in which the egg white was replaced by casein. The animals were fed *ad libitum*. The average food intake from the twentieth to the twenty-ninth days on the egg white containing diet was 2.5 gm. per day per mouse. The average daily intake when these animals were transferred to the basal diet was 3.7 gm. Mitosis occurred irregularly from the second to the sixth days after the transfer with a peak of activity on the sixth day. In experiments 116 and 192 (Fig. 1) in which the deficient mice were also transferred to the basal diet and fed *ad libitum* no extraordinary mitotic activity occurred in the liver, but mitotic figures were present in greatest numbers on the sixth and seventh days after the transfer. In experiment 228 (Fig. 1) the intake of the basal diet was controlled so that the mice received the same amount of the basal diet (av. 2.8 gm. per day per mouse) as they had previously eaten of the diet containing egg white. The daily ration was fed all at once and it was consumed immediately. It seemed to be insufficient to satisfy their appetites. A small amount of mitotic activity was present in the liver after 12 hours and after 5 and 6 days after transfer to this diet.

Injections of biotin were made subcutaneously into biotin deficient mice in 6 experiments. In experiment 139 a group of mice was used that had been placed on an egg white containing diet at 30 days of age and that had developed mild deficiency symptoms in 32 days. After 48 days on the diet when deficiency symptoms were marked, daily injections of 1.5γ of biotin were begun, while the mice continued to receive the egg white diet. Animals were then sacrificed at daily intervals. A small amount of mitotic activity was found on the second day only (Fig. 2). In experiments 143 and 261 (Fig. 2) the biotin deficient mice were given 2γ of biotin daily and in experiment 227 (Fig. 2) a single injection of 10γ of biotin was given. In all of these experiments the effect, though positive, was small, early, and of short duration.

The effect of biotin injections on liver mitosis was also studied in normal animals. Six experiments were conducted using in each 7 to 10 young adult mice, 75 to 111 days of age. In three the animals received 2γ of biotin daily and in three they received a single injection of 10γ of biotin. In experiment 172 mice which were 104 and 106 days of age and which were maintained on laboratory chow were given daily subcutaneous injections of 2γ of biotin. Mitotic activity appeared in the liver of the mouse killed on the third day

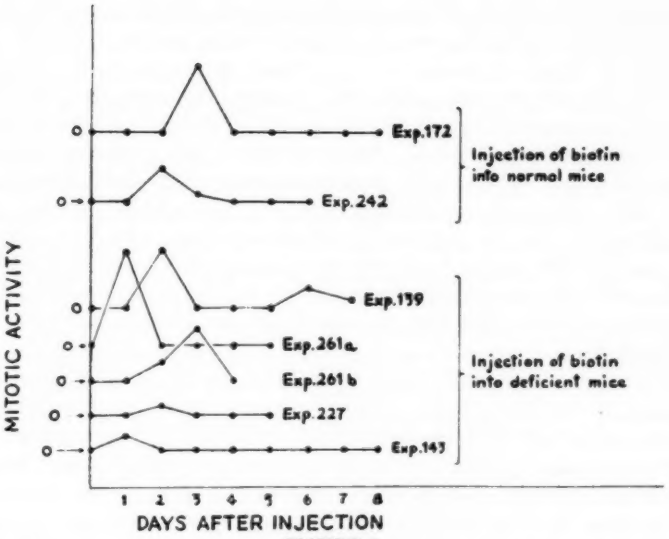

FIGURE 2

Mitotic activity in the livers of normal mice and of biotin deficient mice after the injection of biotin.

of the injection period (Fig. 2). In experiment 242, 70- to 74-day-old mice, maintained on a basal semisynthetic diet containing 10 per cent dried liver which must contain a large amount of biotin, received daily injections of 2γ of biotin. A small amount of mitotic activity was present on the second day (Fig. 2). In the 4 remaining experiments a few mitotic figures appeared in the liver but activity was at most very slight. As in the deficient mice the effect was early, and of brief duration.

Discussion

Biotin is one of the most potent of the growth factors. Extremely small amounts are effective in the growth of the microorganisms that require it and the normal daily requirement for the rat and mouse is of the order of only 2 to 5γ. What part it plays in the multiplication of the animal cell is not known. In our experiments we have found some stimulation when deficient animals are treated with biotin, and some stimulation when biotin is injected into normal, non-

deficient, animals. There have been no spectacular effects, however, and the appraisal of the results requires some caution.

When a group of young mice is placed on a deficiency producing diet, the mitotic activity of the liver persists for some time and then falls off irregularly so that by the time the symptoms appear (after 4 to 5 weeks) no mitosis is found. This is not different, however, from the events in normally growing mice, for mitosis declines in the liver after the fourth week and occurs only sporadically in older mice. Furthermore, as the deficiency symptoms appear the food intake decreases which results in a partial inanition which also brings about a cessation of mitosis, as reported by Leduc (1949). We cannot therefore conclude that the decreased mitotic activity is due to lack of biotin.

When biotin is injected into animals that are biotin deficient mitotic activity in the liver always occurs but it is always small in amount, apears early and is of short duration (Fig. 2). In 6 groups of non-deficient, normal animals similarly injected with biotin a like amount of mitotic activity occurred in two groups and almost none was found in the other 4. The failure to obtain a spectacular increase in mitotic activity in the liver when biotin deficient animals are treated with biotin suggests that under the conditions of dietary deficiency biotin is not a limiting factor in mitosis. There are a number of observations in the literature that corroborate this. West and Woglom (1942) found that tumors produced large healthy growths when transplanted into mice in an extreme stage of biotin depletion. Lazere, Thomson and Hines (1943) found that in rats maintained on an egg white containing diet and showing the characteristic symptoms of biotin deficiency, nerve regeneration and muscle recovery occur at rates comparable to those of control animals. They conclude that if biotin is essential for neuromuscular regeneration, the requirements must be below the amount still available in a deficient animal. Kornberg, Tabor, and Sebrell (1945) found that in biotin deficient rats suffering a progressive decline in weight terminating in death restoration of red blood cells and hemoglobin after repeated hemorrhages compares favorably with that in animals on an adequate diet.

Biotin seems to have little or no effect on wound healing. Bosse and Axelrod (1948) found that the healing of skin ulcers occurred equally rapidly in rats exhibiting severe symptoms of biotin deficiency and in control rats. We have made similar wounds in the skin of

biotin deficient mice and can corroborate the statement that they heal readily.[4] The result is particularly surprising because the skin changes are among the most striking symptoms of this deficiency. From all of these reports it seems clear that in animals that are biotin deficient the capacity of the tissue cells for mitosis is not impaired. It seems obvious, then, that if biotin is essential for mitosis the symptoms of the disease appear before the level of the cells themselves is reduced to the point where mitosis is impossible.

When biotin is injected into normal young adults on the stock diet, which is abundantly adequate in biotin, mitotic activity in the liver is initiated. Although the effect is slight it is positive and it seems significant for no mitosis would ordinarily be found at this age. The effect is, moreover, a fleeting one and cannot be repeated, for with subsequent daily injections it does not recur. Injection of a corresponding amount of distilled water or of Ringer's fluid was found to have no effect. We have considered the possibility that we were dealing with a condition of near deficiency such as, apparently, was present in the experiments of Nielsen and Black (1944) who obtained improved growth in mice on a diet with very low biotin content. In our experiments, the animals were on a stock diet containing an adequate supply of biotin. It is possible, however, that the biotin of the diet is not all available to the animal. It is conceivable that the intestinal bacteria may compete with the animal for the biotin of the diet and render it unavailable so that even in the presence of an apparent abundance, the animal is on the margin of deficiency. We doubt, however, that the increased mitosis we have observed can be explained in this way for we have been unable to obtain any observable increase in weight or growth rate by the injection of biotin. Furthermore, the increased mitotic activity produced in the liver by the injection of biotin is slight and of brief duration. The effect of the injected biotin on the liver cannot be indirect, through changes in the circulation since it is reported to have no pharmacodynamic effect (Schmidt and Landy, 1942). It would seem then to be a direct effect on the liver cells themselves.

The effect of biotin on isolated cells has been studied in cultures of yeast and bacteria, but its effect on tissue cells of higher animals has been little studied. Hamilton and Plotz (1942) have reported

[4]These observations were made in connection with a study of the skin of biotin deficient mice being conducted by Mr. Harold Rauch of this department.

that it has a stimulating effect on the growth of nerve tissue *in vitro*. They comment on the fact that the medium must have contained considerable biotin to begin with since it contained chick embryo extract. It is therefore surprising that such a slight addition should have an effect. Furthermore active proliferation of cell bodies was observed only in the original cultures and not in subsequent subcultures. Since no evidence of mitotic activity was found, this proliferation is attributed to amitosis. Burt (1943) failed to corroborate these observations and questioned the evidence for cell proliferation. However in two respects the observations of Hamilton and Plotz are like our own on the liver cells of the intact organisms. In the presence of an apparently adequate supply of biotin the addition of a small amount of pure biotin produces a brief increase in cell proliferation which subsides and does not recur. The effect may be due to the immediate availability of the pure biotin. Much of the biotin in tissues and elsewhere is in a combined form from which it is liberated only with difficulty. It is suggestive that the liver cells of animals with extreme symptoms of deficiency still contain relatively large amounts of biotin. West and Woglom (1942) report levels as high as 0.58γ per gram of fresh liver in deficient mice as compared with 3.12γ for normal livers and Wright and Welch (1944), 0.35γ per gram in livers of deficient rats as compared with 1.5γ per gram for normal ones. West and Woglom further report that mice are capable of tolerating enormous doses of biotin either fed or injected without any change in the free or total biotin content of the liver. From these observations we would tentatively conclude that biotin is necessary for mitotic division of the liver cells, that the biotin already present in the tissues and tissue fluids is not as readily available as pure biotin, and that the cell can respond to only a limited amount of additional pure biotin.

Our general conclusion is then that although biotin does effect mitotic activity in the liver cell, this effect is slight even in animals that have developed severe symptoms on a biotin deficient diet. This seems to be due to the fact that the animal will die before the biotin supply of the tissues is lowered to a point where normal mitotic activity is impossible and that the cure of the deficiency involves other factors, such as increased food intake and more efficient food utilization, that in their effect tend to obscure the direct effect of biotin

itself. Apparently the effect of the biotin is always slight and fleeting and not commensurate with the size of the dose. A dose as high as 100γ was found to produce no striking effect.

In comparison with injected liver, previously reported, the effect of the biotin is always earlier and much less. The timing might be attributed to the fact that the biotin of the liver is in combination from which it must first be liberated. However, if West and Woglom's (1942) figures are approximately correct, the biotin content of the liver injected in our experiments would be about 2γ per mouse, but the effect of the injected liver exceeds that obtained with any amount of biotin. We conclude, therefore, that the stimulating effect of injected liver on the mitotic activity of the liver is due to something besides its biotin content.

Summary

1. Mitotic activity has been studied in the livers of mice during the development of biotin deficiency and during treatment by injection of biotin or by adjustment of the diet. Mitotic activity declined during the development of the deficiency and was stimulated by treatment, but no effects were found that could not be attributed to other factors than biotin.
2. A slight stimulation of mitotic activity was found in the livers of normal mice after subcutaneous injection of biotin. This is attributed to a direct effect of pure biotin even in the presence of an abundance of biotin in combined form.
3. It is suggested that under the condition of dietary deficiency the biotin content of the tissues does not fall so low that it is insufficient to support cell division.
4. The effect of injected liver in stimulating mitotic activity in the liver, previously reported, must be due to something other than its biotin content.

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VARIATIONS IN BODY WEIGHT AND ORGAN : BODY-WEIGHT RATIOS OF INBRED LINES OF WHITE LEGHORN CHICKENS IN RELATION TO MORTALITY, ESPECIALLY FROM LYMPHOMATOSIS[1]

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The extreme variation between individuals in respect to weight of the entire body and also of individual organs is well known and is common for all animals; the causes of this variability, however, are not so well understood. Within any one species, age, sex, amount and type of diet, exposure to disease, genetic constitution, and various interactions of these basic factors undoubtedly are all important in determining the size characteristics of the individual. No attempt will be made to review completely the literature on this subject. Attention will be directed primarily to the consideration of intra-species variation in organ:body-weight rations between individuals of comparable age and body weight.

In general, liver and pancreas weights have been found to be more closely related to body weight than is spleen weight. Growth of the total body and of the organs as observed for the guinea pig (Bessesen and Carlson, 1; Eaton, 10), the cat (Latimer, 24, 25), the rat (Hatai, 14; Jackson, 19; Donaldson, 7, 8; Cameron, 5; Hammett, 13; Freudenberger, 11, 12; Moment, 37; Webster, *et al.*, 47), the sparrow (Quiring and Bade, 39), the pigeon (McCarrison, 31, 32, 33; Riddle, 41), the turkey (Latimer and Rosenbaum, 26; Marsden, 34),

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and the chicken (Souba, 42; Latimer, 23; Mitchell, *et al.*, 35, 36; Juhn and Mitchell, 20; Hopkins and Biely, 17; Landauer and Aberle, 22, Breneman, 2; Oakberg, 38) is remarkably uniform. This uniformity of different species in growth pattern has been observed by Welcker and Brandt (48) and by Brody and Kibler (4). Within any one species, the highest organ:body-weight ratios have been observed in young individuals, with a decline in relative organ weights during adolescence and maturity. (An exception to this pattern is, of course, the gonads and especially the ovary of birds.) Marked variation in liver:body-weight and pancreas:body-weight ratios has been observed, and the spleen has been found to be one of the most variable of the body organs.

Differences in organ weights and organ:body-weight ratios in Laboratory animals attributable to genetic constitution have been described by Hatai (15, 16), Donaldson (8), Riddle (40, 41), King and Donaldson (21), Landauer and Aberle (22), Leonard and Righter (27), Lerner (28), Strandskov (43), Eaton (10), and Dunn, *et al.* (9). Differences in organ weights between inbred lines of guinea pigs have been ascribed to general growth factors affecting the organism as a whole (Eaton, 10). In relation to the skeleton of the rabbit and the chicken, a combination of general growth factors which are of primary importance with secondary factors which are specific for certain bones and also for homologous groups of bones has been indicated (Wright, 49, 50).

To our knowledge, no attempt has been made to associate differences in organ:body-weight ratios of chickens with degree of resistance or susceptibility to disease. These comparisons cannot yield definite answers, for it is apparent that many factors operate in determining both organ weight and host reactions to pathogenic agents. However, any associations with disease incidence observed in this material (particularly lymphomatosis) may aid in selection of further problems on the physical bases for resistance of the host.

Materials and Methods

Three hundred eighty adult females, approximately 600 days old, and in apparently good physical condition were used. These individuals were the survivors from the H (1946) population, and were distributed among 13 inbred lines and 81 families. As here used,

family refers to progeny from a single sire and dam. The breeding plan and general procedure for this material were the same as those given by Waters (45). However, there are discrepancies in the designation of direction of selection as reported by Waters (45), and Waters and Prickett (46). The classification used here is given in the Eighth Annual Report of the Regional Poultry Research Laboratory (44) and agrees with that of Waters and Prickett (46).

The birds were weighed, then killed by electrocution. Liver, pancreas, and spleen were removed immediately, blotted to remove excess blood and peritoneal fluid, and weighed. Organ weights from individuals with grossly visible lymphomatosis were not used.

Mortality of sibs of the survivors prior to termination of the experiment was obtained from the Laboratory records. All individuals with grossly visible lymphoid tumors were classified as dead from lymphomatosis even though other pathology may have been present. Also, all birds with gross manifestation of lymphomatosis when killed at 600 days were classed as lymphomatosis positive. This results in a bias in the data, but the difficulty of determining the actual cause of death makes such procedures justifiable, especially when incidence of lymphomatosis is the primary objective. Autopsied birds with pathology other than lymphomatosis were grouped as "other deaths."

Comparisons of incidence of deaths from lymphomatosis and "other deaths," incidence of mortality and body weight, and incidence of mortality and organ:body-weight ratios were based only on families of 8 or more birds. This procedure avoids the more extreme discontinuities arising from percentages based on small numbers. For lines 5 and 14, only one family per line was represented by more than 8 birds; as this would result in complete confounding of families and lines, these lines also were omitted.

Results

Total Body Weight and Organ Weights

Gross Weights.—Live body weight, weights of the liver, pancreas, and spleen, and organ:body-weight ratios are given by families and lines from the third through the ninth column in Table 1. Marked differences appear between lines and between families within lines in total body weight and in organ weights. Variance analysis indicated highly significant differences between lines and between families within lines

TABLE 1

LINE AND FAMILY MEAN BODY, LIVER, PANCREAS, AND SPLEEN WEIGHTS (IN GRAMS), ORGAN:BODY-WEIGHT RATIOS, AND MORTALITY TO 600 DAYS. H (1946) POPULATION

	Family	Body weight	Liver weight	Liver wt. / Body wt. ×100	Pancreas weight	Pancreas wt. / Body wt. ×100	Spleen weight	Spleen wt. / Body wt. ×100	No. of survivors	No. dead with lympho.	No. of "other deaths"	Total population
Line 1	2	2174	40.2	1.85	4.41	.20	3.02	.14	8	4	11	23
	4	1872	34.3	1.83	3.43	.18	3.13	.17	2	4	2	8
	5	1996	39.1	1.96	4.09	.20	2.14	.11	4	6	8	18
	9	1881	39.3	2.09	3.89	.21	2.98	.16	5	1	3	9
	17	1825	49.2	2.70	6.28	.34	3.91	.21	1	13	3	17
Line means		2017	39.6	1.96	4.21	0.21	2.89	0.14	20	28	27	75
Line 2	51	1844	44.2	2.40	2.97	.16	2.21	.12	3	7	12	22
	64	2260	48.2	2.13	3.35	.15	1.62	.07	1	2	3	6
	67	1989	51.6	2.59	3.08	.15	2.98	.15	2	9	8	19
	71	1730	37.7	2.18	2.47	.14	2.37	.14	4	3	6	13
	72	2273	58.6	2.58	4.09	.18	2.11	.09	2	10	8	20
Line means		1936	46.0	2.37	3.04	0.16	2.33	0.12	12	29	34	80
Line 3	106	1726	29.5	1.71	2.18	.13	2.27	.13	5	4	6	15
	111	1349	26.8	1.99	1.78	.13	3.43	.25	7	8	6	21
	113	1715	36.4	2.12	2.57	.15	2.71	.16	1	1	10	12
	114	1498	34.2	2.28	2.40	.16	3.82	.25	6*	5	5	16
	117	1425	29.3	2.06	2.12	.15	5.10	.36	1	1	8	10
	122	1538	28.1	1.83	1.87	.12	4.00	.26	9	2	4	15
Line means		1520	29.5	1.94	2.03	0.13	3.51	0.23	29	21	39	89
Line 4	154	2160	42.2	1.95	4.31	.20	2.46	.11	1	12	4	17
	161	1890	28.4	1.50	2.91	.15	1.55	.08	1	6	2	9
	162	1988	37.8	1.90	4.21	.21	2.39	.12	4	7	4	15
	165	1610	34.4	2.14	3.07	.19	1.90	.12	1	8	4	13
	172	2128	44.7	2.10	4.28	.20	2.01	.09	6	1	2	9
	174	1814	39.4	2.17	4.11	.23	1.46	.08	5	7	4	16
	175	1737	33.8	1.95	2.76	.16	1.30	.07	3	3	4	10
Line means		1936	39.2	2.02	3.89	0.20	1.84	0.10	21	44	24	89

TABLE 1 (*continued*)

	Family	Body weight	Liver weight	Liver wt. / Body wt. ×100	Pancreas weight	Pancreas wt. / Body wt. ×100	Spleen weight	Spleen wt. / Body wt. ×100	No. of survivors	No. dead with lympho.	No. of "other deaths"	Total population
Line 5	201	2063	53.7	2.60	3.39	.16	4.23	.20	2	3	2	7
	203	2115	51.3	2.43	3.37	.16	2.15	.10	1	0	1	2
	206	1876	47.4	2.53	3.49	.19	4.49	.24	7	3	4	14
	207	2258	46.6	2.06	4.23	.19	2.32	.10	2	0	3	5
Line means		1991	48.6	2.44	3.59	0.18	3.89	0.20	12	6	10	28
Line 6	254	2137	43.3	2.02	2.84	.13	2.69	.13	9	2	3	14
	255	2135	39.4	1.85	3.28	.15	2.86	.13	16	2	6	24
	257	1928	35.1	1.82	2.81	.15	2.40	.12	7*	1	3	11
	263	1758	31.0	1.76	2.62	.15	2.29	.13	9	1	2	12
	266	1876	32.6	1.74	2.70	.14	2.41	.13	8	1	3	12
	267	2031	48.2	2.37	3.01	.15	2.61	.13	10	2	5	17
	268	2047	36.5	1.78	2.62	.13	2.29	.11	6	1	4	11
	269	2079	38.5	1.85	2.89	.14	3.19	.15	7	1	8	16
	270	1873	37.6	2.00	2.86	.15	2.68	.14	5	2	2	9
	273	1831	35.1	1.92	2.89	.16	2.16	.12	3	2	6	11
Line means		1997	38.4	1.92	2.90	0.15	2.61	0.13	80	15	42	137
Line 7	302	2089	37.7	1.80	3.02	.14	3.19	.15	15	6	2	23
	304	2105	41.8	1.99	3.11	.15	3.04	.14	10	7	5	22
	305	2198	38.6	1.75	2.86	.13	2.93	.13	7	3	4	14
	306	2121	39.6	1.87	3.34	.16	4.06	.19	6	11	3	20
	308	2109	41.4	1.96	3.34	.16	3.35	.16	10	3	4	17
	310	2408	54.1	2.25	3.90	.16	3.62	.15	6	4	4	14
	315	2137	43.9	2.05	3.31	.16	3.58	.17	8	2	6	16
	316	2089	39.5	1.89	2.90	.14	3.45	.17	9	6	6	21
	317	2001	37.5	1.88	2.87	.14	2.81	.14	11	2	6	19
	318	1849	34.4	1.86	2.59	.14	2.64	.14	11	1	2	14
Line means		2089	40.1	1.92	3.07	0.15	3.20	0.15	93	45	42	180

TABLE 1 (*continued*)

	Family	Body weight	Liver weight	Liver wt. / Body wt. ×100	Pancreas weight	Pancreas wt. / Body wt. ×100	Spleen weight	Spleen wt. / Body wt. ×100	No. of survivors	No. dead with lympho.	No. of "other deaths"	Total population
Line 9	401	1590	41.6	2.61	3.07	.19	2.89	.18	2	8	5	15
	409	1694	37.7	2.22	3.01	.18	2.08	.12	4	9	9	22
	410	1385	31.7	2.29	2.48	.18	1.84	.13	1	6	5	12
	411	1862	44.0	2.36	4.07	.22	1.93	.10	5	8	9	22
	421	1530	30.2	1.97	2.39	.16	2.02	.13	2	4	5	11
	426	1843	47.8	2.59	3.36	.18	3.03	.16	3	6	5	14
Line means		1720	40.5	2.36	3.29	0.19	2.27	0.13	17	41	38	96
Line 10	470	1842	38.1	2.07	3.75	.20	3.95	.21	2	0	3	5
	471	1711	43.8	2.56	3.75	.22	2.52	.15	6	3	7	16
	473	1499	34.0	2.26	3.23	.22	3.21	.21	2	5	1	8
	475	1906	46.9	2.46	3.79	.20	2.25	.12	12	3	3	18
	476	2168	41.1	1.90	4.04	.19	3.04	.14	1	0	0	1
	478	2267	95.9	4.23	4.11	.18	2.74	.12	1	1	3	5
	482	1643	38.3	2.33	3.69	.22	3.13	.19	1	2	3	6
Line means		1836	45.8	2.49	3.75	0.20	2.61	0.14	25	14	20	59
Line 11	527	2224	49.8	2.24	6.13	.28	1.84	.08	2	5	16	23
	534	1550	37.8	2.44	3.94	.25	1.72	.11	2	4	6	12
	538	1697	45.8	2.70	3.41	.20	1.97	.12	1	1	0	2
	540	2585	75.0	2.90	4.09	.16	1.67	.06	1	3	1	5
	542	2306	47.4	2.06	4.54	.20	2.16	.09	3	4	11	18
Line means		2083	48.7	2.34	4.58	0.22	1.92	0.09	9	17	34	60

TABLE 1 (*continued*)

	Family	Body weight	Liver weight	Liver wt. / Body wt. ×100	Pancreas weight	Pancreas wt. / Body wt. ×100	Spleen weight	Spleen wt. / Body wt. ×100	No. of survivors	No. dead with lympho.	No. of "other deaths"	Total population
Line 13	613	2013	39.2	1.95	3.19	.16	2.40	.12	5	6	8	19
	615	2348	56.1	2.39	4.03	.17	2.08	.09	6	2	5	13
	616	2283	51.2	2.24	3.82	.17	2.00	.09	8	6	6	20
	617	2075	47.9	2.31	3.87	.19	2.76	.13	2	5	5	12
	621	2056	49.4	2.40	3.79	.18	1.88	.09	5	6	6	17
	624	2055	43.0	2.09	4.08	.20	2.55	.12	7	4	6	17
Line means		2159	48.0	2.23	3.81	0.18	2.22	0.10	33	29	36	98
Line 14	653	2065	68.6	3.32	3.76	.18	2.41	.12	1	1	2	4
	655	1240	46.9	3.78	2.07	.17	0.94	.08	1	2	3	6
	656	2076	53.2	2.56	3.97	.19	2.63	.13	6	3	2	11
	660	1195	49.1	4.11	3.02	.25	2.05	.17	1	0	1	2
Line means		1884	53.8	2.85	3.63	0.19	2.35	0.12	9	6	8	23
Line 15	701	1510	36.0	2.38	2.82	.19	2.72	.18	2*	11	6	19
	704	1653	33.5	2.03	2.66	.16	2.67	.16	4	7	7	18
	706	1875	55.3	2.95	3.33	.18	1.89	.10	1	10	5	16
	708	1833	41.5	2.26	2.87	.16	2.16	.12	5	5	5	15
	718	1681	47.8	2.84	3.02	.18	1.82	.11	4	2	5	11
	721	1683	36.1	2.14	2.49	.15	1.90	.11	7	4	7	18
Line means		1712	39.8	2.33	2.76	0.16	2.12	0.12	23	39	35	97
		Total, Mortality								334	389	1111
		Mortality, families of 8 or more only (lines 5 and 14 omitted)								315	361	1030

*One survivor with no weights.

in respect to total body, liver, and pancreas weights. Differences between lines in spleen weight were highly significant, with no detectable differences between families within lines.

The relationship of organ weights and body weight was determined by the correlation coefficients shown in Table 2. Pancreas and liver

TABLE 2
CORRELATION COEFFICIENTS FOR ORGAN-BODY WEIGHT AND INTER-ORGAN COMPARISONS

	Degrees of freedom	Spleen weight	Pancreas weight	Liver weight
Body weight				
Total	378	—0.052	0.482**	0.482**
Lines	11	—0.045	0.501	0.492
Families within lines	67	0.113	0.643**	0.666**
Within families within lines	298	—0.110	0.390**	0.397**
Liver weight				
Total	378	—0.075	0.608**	
Lines	11	—0.371	0.723**	
Families within lines	67	0.105	0.563**	
Within families within lines	298	—0.040	0.601**	
Pancreas weight				
Total	378	—0.105*		
Lines	11	—0.446		
Families within lines	67	0.231		
Within families within lines	298	—0.037		

*Significant at 5% level.
**Significant at 1% level.

weights show highly significant correlations with body weight for the total population, families within lines, and within families. Spleen weight tends to show a negative correlation with body weight, but the r values are so low as to be considered unimportant. As expected, inter-organ correlations were high between pancreas and liver. Spleen weight gave negative r values with liver and with pancreas weights for the total population, lines, and within families, and a positive correlation on the family basis; however, all values are so low that no relationship is indicated except for the r of —0.105 between pancreas and spleen for the total population.

Organ : Body - Weight Ratios.—Examination of the organ:body-weight ratios given in Table 1 suggested line differences. This observation was substantiated by covariance analysis where highly significant line differences are indicated for liver, pancreas, and spleen weights after correction for body weight. Differences between families are greatly reduced by correction for body weight, with no significance

for liver weight and a reduction of significance from the 1 to the 5 per cent level for pancreas weight. Spleen weight still shows no differences between families within lines with practically no change from the variances before correction for body weight. These results would be expected on the basis of correlations given in Table 3.

TABLE 3
CORRELATION COEFFICIENTS FOR BODY-WEIGHT AND ORGAN:BODY-WEIGHT RATIOS

	Degrees of freedom	Spleen wt. / body wt.	Pancreas wt. / body wt.	Liver wt. / body wt.
Body weight				
Total	378	—0.353**	—0.137**	—0.145**
Lines	11	—0.531	0.030	—0.245
Families within lines	67	—0.305**	—0.126	0.005
Within families within lines	298	—0.326**	—0.282**	—0.160**
Liver weight/body weight				
Total	378	0.081	0.526**	
Lines	11	—0.127	0.606*	
Families within lines	67	0.063	0.356**	
Within families within lines	298	0.145**	0.552**	
Pancreas weight/body weight				
Total	378	0.031		
Lines	11	—0.376		
Families within lines	67	0.256**		
Within families within lines	298	0.182**		

*Significant at 5% level.
**Significant at 1% level.

The correlation coefficients given in Table 3 were calculated as an estimate of the tendency of heavy individuals to have lower organ:body-weight ratios. The highly significant negative correlations obtained for the total populations and for birds within families support this hypothesis. The r value based on families also was highly significant for the spleen and body weight comparison. Inter-organ comparisons are interesting; the high degree of association of pancreas and liver ratios was expected. Significant correlations within families for spleen:body-weight with liver: and pancreas:body-weight ratios indicate common size-determining factors that are independent of body weight. Families also showed a significant correlation for the spleen:body-weight and pancreas:body-weight ratios. With the exception of the liver-pancreas comparisons, these correlations result primarily from variations within families. As this is within the most genetically homogeneous units, environmental factors appear to be

the most probable cause of negatve correlations between body-weight and organ:body-weight ratios.

Mortality to 600 Days

Line Differences.—In Table 4 the different inbred lines are listed in order of increasing susceptibility to lymphomatosis as measured by gross diagnosis of all birds dying from 30 to 600 days. These data are from the H (1946) population of the genetic flock. Only families with 8 or more birds and with at least one survivor were used; therefore, these percentages may not agree with those published elsewhere. Percentage of mortality from lymphomatosis, from "other deaths," and total mortality varied greatly, and line differences were highly significant by the chi-square test (Table 4).

Families Within Lines.—Probabilities of homogeneity of families within lines (chi-square) are given in Table 5. Some lines, especially 2, 6, 9, 11, and 13, are remarkably uniform both for mortality from lymphomatosis and from "other deaths." The reliability of this test may be questioned, however, for incidence of mortality undoubtedly

TABLE 4
Mortality in the Different Lines Through 600 Days After Hatching. Families of 8 or More Birds Only

Line	Direction of selection*	No. of families per line	Total population	No. dead with lymphomatosis	% Dead with lympho.	No. dead from "other causes"†	% Dead from "other causes"	Total No. dead	% Total mortality
6	R	10	137	15	11	42	31	57	42
3	R	6	89	21	24	39	44	60	67
7	S	10	180	45	25	42	23	87	48
11	S	3	53	13	25	33	63	46	87
10	R	3	42	11	26	11	26	22	52
13	R	6	98	29	30	36	37	65	66
1	R	5	75	28	37	27	36	55	73
2	S	4	74	29	39	34	46	63	85
15	S	6	97	39	40	35	36	74	76
9	S	6	96	41	43	38	40	79	82
4	S	7	89	44	49	24	27	68	76
Totals			1,030	315	31	361	35	676	66
X^2, Line differences in mortality				60.94		41.16		108.62	
P				<.01		<.01		<.01	

*R = Selection for resistance to lymphomatosis, S = Selection for susceptibility to lymphomatosis.

†Birds in which no lymphoid tumors were visible at autopsy.

TABLE 5
PROBABILITIES OF HOMOGENEITY OF FAMILIES WITHIN LINES IN RELATION TO CAUSES OF DEATH AS DETERMINED BY CHI-SQUARE

Line	No. of families	Per cent with lympho-matosis	Per cent lympho-matosis (corrected for O.D.)	Per cent "Other deaths"	Per cent Total mortality
6	10	0.97	0.91	0.49	0.48
3	6	0.26	0.62	<0.01**	0.06
7	10	0.05*	0.08	0.59	0.22
11	3	0.72	0.85	0.52	0.70
10	3	0.04*	0.07	0.13	0.09
13	6	0.74	0.50	0.98	0.58
1	5	<0.01**	<0.01**	0.30	0.08
2	4	0.34	0.23	0.79	0.36
15	6	0.06	0.03*	0.97	0.13
9	6	0.91	0.87	0.99	0.15
4	7	0.07	<0.01**	0.97	0.02*

*Probability of 0.05-0.02.
**Probability of 0.01 or less.

affects the variation. Therefore, more emphasis has been placed on those lines which show significant inter-family variation in mortality. Correction of the total population for deaths from "other causes" (Method II, Lucas and Oakberg, 29) resulted in no marked shifts in the P values obtained for family differences in percentage of lymphomatosis except for line 4. The use of the "uncorrected" population probably is preferable, for larger numbers are available. Family differences in percentage of lymphomatosis are indicated for lines 1, 7, and 10. Line 3 was the only case where mortality due to "other causes" varied significantly, and for total mortality, line 4 alone showed significant inter-family variation.

Association of Deaths from Lymphomatosis and from "Other Causes."—Total mortality could be ascribed to 46.60 per cent from lymphomatosis and 53.40 per cent from "other causes." Chi-square comparison showed that the probability was less than 0.001 that the ratios of cause of death in the different lines were uniform. However, when the analysis was based on lines 1, 2, 3, 7, 9, 10, 13, and 15, the probability was 0.62. Thus for these lines, representing a range in total mortality from 48 to 85 per cent, a correlation of incidences of lymphomatosis and other causes of death is indicated. Lines 4, 6, and 11 do not show the same ratio between causes of death as do the other lines, with an excess of "other deaths" in lines 6 and 11 and a preponderance of deaths from lymphomatosis in line 4.

Mortality by 100-Day Periods.—The stage in the life cycle of the population at which the organ weights were taken and the experiment on resistance and susceptibility terminated may influence comparison of these variables. The resulting bias would be minimized if death rate attributable to the different causes were constant throughout the population. Mortality is given by 100-day periods from 100 to 600 days in Table 6. Survivors with lymphomatosis were not included in

TABLE 6
PERCENTAGE OF MORTALITY BY 100 DAY PERIODS FROM 100 TO 600 DAYS

Age in days at death	Deaths from lympho-matosis	Deaths from lympho-matosis* corrected for "other deaths"	"Other deaths"	"Other deaths"* corrected for lympho-matosis	Total mortality
101-200	5.2	5.8	9.7	10.3	14.9
201-300	9.6	10.5	8.1	9.0	17.8
301-400	10.2	10.9	6.4	7.2	16.7
401-500	7.0	8.1	12.7	13.6	19.7
501-600	3.9	4.5	12.2	12.8	16.2
X^2 Values for period differences	28.928	26.149	19.574	17.134	6.314
P Values	<0.01	<0.01	<0.01	<0.01	0.18

*Method II, Lucas and Oakberg, in press.

estimation of the 501-600 day group. Percentages based on the total surviving population at the beginning of each period differed but slightly from results obtained when correction for "other deaths" was made for percentage of deaths from lymphomatosis and when correction for deaths from lymphomatosis was made for percentage of "other deaths" (Method II, Lucas and Oakberg, 29). The latter method of course gives higher percentages in each case, but the pattern for the age groups is not altered. Total mortality was fairly constant, but incidence of deaths from lymphomatosis was low from 101-200 days, at a peak from 201-400 days, then declined from 401-600 days. Mortality from "other causes" was highest in the 401-600 day period. Thus, at the time the organ weights were taken, lymphomatosis mortality was on the decline while "other deaths" were at a peak. These results differ from those reported by Lucas and Oakberg (29), where a constant rate of deaths from lymphomatosis was observed throughout the 100-600 day period. However, the incidence of 5 per cent

TABLE 7

PROBABILITIES OF HOMOGENEITY IN MORTALITY FOR 100 DAY PERIODS ON A LINE BASIS AS ESTIMATED BY CHI-SQUARE (FAMILIES OF 8 OR MORE BIRDS ONLY, 100 TO 600 DAYS)

Line	Deaths from lymphomatosis	"Other deaths'	Total mortality
1	0.77	0.43	0.82
2	0.29	0.11	0.08
3	0.10	0.34	0.02*
4	0.03*	0.31	0.06
6	0.71	0.31	0.70
7	0.02*	0.25	0.28
9	0.22	0.15	0.21
10	0.38	0.26	0.08
11	0.10	0.14	0.04*
13	<0.02*	<0.01**	0.04*
15	0.28	<0.02*	0.63

*Probability of 0.05 to 0.02.
**Probability of 0.01 or less.

lymphomatosis among the survivors was higher than the 4 per cent for the G (1945) population (Lucas and Oakberg, 29).

Comparison of mortality within lines indicated significant differences in lymphomatosis mortality by periods for lines 4, 7, and 13 (Table 7). Period differences for "other deaths" were indicated for lines 13 and 15, and for total mortality in lines 3, 11, and 13. Thus it appears that differences indicated in Table 6 are the cumulative effect of mortality curves characteristic for each line; shape of the composite curve will be determined largely by the distribution of the total population among the different lines. While some reservation is necessary, analysis of age at death within lines indicates that the influence of age is less than would be indicated by measurement of the total population. This is especially true of comparisons made on line and family bases.

RELATIONSHIP OF PERCENTAGE OF MORTALITY WITH ORGAN:BODY-WEIGHT RATIOS

Correlation coefficients for percentage of mortality, body-weight, organ:body-weight ratios, and spleen weights are given in Table 8. The results obtained by basing percentage of deaths from lymphomatosis on the total population and on the population corrected for other deaths (Method II, Lucas and Oakberg, 29) were almost identical. Use of the "total population" probably gives better results,

as number of birds per family and line is larger; unless otherwise specified, percentages were calculated by this method.

Organ:Body-Weight Ratio and Percentage of Deaths from Lymphomatosis.—Percentage of lymphomatosis mortality was positively correlated with liver:body-weight ratio on the basis of the total population, but not between families within lines (Table 8). Higher *r*

TABLE 8

CORRELATION COEFFICIENTS, BODY WEIGHT, SPLEEN WEIGHT, LIVER:BODY-WEIGHT RATIO, AND PANCREAS:BODY-WEIGHT RATIO WITH PERCENTAGE OF MORTALITY

	Degrees of freedom	Body weight	Liver wt. / Body wt.	Pancreas wt. / Body wt.	Spleen weight
Percentage mortality from lymphomatosis					
Total	64	—0.203	0.329**	0.413**	—0.081
Lines	9	—0.252	0.524	0.507	—0.496
Families within lines	54	—0.162	0.174	0.339*	0.305*
Percentage mortality from lymphomatosis (Corrected for other deaths)					
Total	64	—0.261*	0.418**	0.445**	—0.157
Lines	9	—0.352	0.658*	0.525	—0.513
Families within lines	54	—0.150	0.173	0.336*	0.267*
Percentage "other deaths"					
Total	64	—0.126	0.175	0.075	—0.089
Lines	9	—0.364	0.419	0.184	—0.110
Families within lines	54	0.113	—0.029	—0.062	—0.071
Percentage total mortality					
Total	64	—0.274*	0.419**	0.415**	—0.139
Lines	9	—0.431	0.667*	0.500	—0.447
Families within lines	54	—0.073	0.156	0.286*	0.243

*Significant at 5 per cent level.
**Significant at 1 per cent level.

values were obtained in the pancreas:body-weight comparisons, with high significance for the total *r* and a 5 per cent level of significance for families within lines. Spleen weight showed a significant positive correlation of 0.267 between families within lines. Body weight gave negative *r* values, but the values are too small to be significant.

Organ:Body-Weight Ratios and Percentage of "Other Deaths."—"Other deaths" gave significant correlations neither with body-weight, spleen-weight, nor organ:body-weight ratios.

Organ:Body-Weight Ratios and Percentage of Total Mortality.—Correlations obtained for total mortality represent the pool of lymphomatosis and "other deaths"; most of the significance can be

attributed to the effect of lymphomatosis. The significant negative correlation, —0.274, for body weight probably resulted primarily from deaths from lymphomatosis with a smaller contribution from "other deaths." Correlation with liver:body-weight and pancreas:body-weight ratios was due almost entirely to the effect of lymphomatosis.

Discussion

A constant relation of weight of parts to the whole would be expected on the basis of conclusions given by Castle (6) for the rabbit, by Eaton (10) for the guinea pig, and by Lerner (28) for the chicken. Lerner did observe a different growth pattern in bantam fowls than in the Minorca and Plymouth Rock. Our data on inbred lines of White Leghorn chickens indicate common growth factors for total body, liver and pancreas. However, specific factors for liver and pancreas also are indicated, and the close correlation of these organs suggests response to a common mechanism. Spleen weight was independent of body, liver, and pancreas weights; the relationship of this difference to growth of other lymphoid tissues will be subjected to further study. Thus, the results for the liver and pancreas of the chicken parallel those demonstrated for growth of the rabbit skeleton given by Wright (49) and for the chicken skeleton (Wright, 50) where general growth factors were considered to be of primary importance, with secondary factors specific for certain bones and for homologous groups of bones.

Liver weight showed more variation than pancreas weight, and consequently gave lower correlations in all comparisons. This variability of the liver is interpreted as due primarily to response to environmental stimuli and greater errors in weighing rather than to basic differences in growth regulation of liver and pancreas.

All r values for the total population and for families within lines indicated a significant negative association of organ:body-weight ratios with body weight. As these individuals were 600 days old, it is unlikely that such differences reflect differences in stages of the growth cycle. Fat storage may be a genetic characteristic differentiating families and lines. Also, emaciation or organ enlargement during preclinical stages of disease may be of importance in raising the organ:body-weight ratio. The latter is suggested by the negative correlation of body weight with total mortality. However, constitutional metabolic patterns may be responsible for differences both in regard to relative organ size and resistance to disease.

Considerable variation in body and organ weights, organ:body-weight ratios, and in mortality exists between families within lines. This type of variation was observed by Briles and McGibbon (3) for blood groups in lines 1 and 2, and in relation to mortality (Lucas and Oakberg, 29). The present lack of information concerning the mode of inheritance of resistance or susceptibility to lymphomatosis precludes interpretation of the apparent heterozygosity within some lines. Present breeding methods may favor heterozygotes in selection for both resistance and susceptibility. Also, low heritability of resistance to lymphomatosis (Lush *et al.*, 30) may be the basic difficulty in increasing uniformity within all lines.

As the birds used for determination of body and organ weights were survivors from a population in which deaths from lymphomatosis and other causes have been common, it may be argued that they represent selected individuals. Mortality by 100 day periods (Tables 6 and 7) gives some support for this criticism. However, this should tend to minimize rather than accentuate group differences, for only the more resistant individuals from the more susceptible lines should live to 600 days. Correlations given therefore may be expected to be underestimated.

The association of incidence of deaths from lymphomatosis and "non-specific" mortality has been observed by Waters (45), Hutt and Cole (18), Lush, *et al.* (30), and Lucas and Oakberg (29). Analysis of the H (1946) population of the Laboratory revealed that a correlation of deaths from lymphomatosis and "other causes" existed for 8 lines. Failure of 3 lines to show the more general parallel incidence of lymphomatosis and "other deaths" may be due to sampling errors or to interactions with environmental factors peculiar to this population of birds. This discrepancy also could arise from different genetic constitutions of the lines. The behavior of 8 of the lines was such as to indicate either common resistance factors both for lymphomatosis and "other causes," or that a significant proportion of "other deaths" should be classified as lymphomatosis (Lucas and Oakberg, 29). This latter circumstance could arise either by manifestations of lymphomatosis other than by grossly visible tumors or to inability at autopsy to detect lymphoid acumulations in the central nervous system and other vital centers of function of the individual.

The correlation of percentage of lymphomatosis with liver: and pancreas:body-weight ratios may be explained by a variety of mecha-

nisms. However, the most plausible hypothesis appears to be that the genetic constitution and thereby the metabolic pattern of the individual is manifested both as an increase in liver and pancreas size and as an increase in susceptibility to lymphomatosis. The conclusion of Brody and Kibler (4) that the neuro-endocrine system is the limiting factor in all metabolism, and the success of Riddle and his associates (41) in developing "endocrine" races of doves and pigeons suggest that the endocrine system may be an important factor in differences we have observed in our material.

Summary

1. Body, liver, pancreas, and spleen weights were taken for 380 apparently normal 600-day old hens representing 13 different inbred lines. Organ weights and organ:body-weight ratios were compared with body weight. Mortality in these lines prior to 600 days was analyzed in relation to differences between families and lines, and in relation to body weight and organ:body-weight ratios.

2. Growth factors were divisible into those which affect the organism as a whole, those which are specific for liver and pancreas, and those which are specific for the spleen. Genetic basis for all factors was indicated by comparison of lines and families within lines.

3. Variation in mortality due to lymphomatosis and to "other causes" was large between lines, and in some cases between families within lines. A correlation of incidence of deaths from lymphomatosis and "other deaths" was indicated for 8 lines. In three lines the ratio of deaths from lymphomatosis to other causes of death deviated significantly from the population average.

4. Positive correlations of incidence of mortality from lymphomatosis with liver: and pancreas:body-weight ratios were interpreted as due to common factors which result both in large pancreas and liver and in susceptibility to lymphomatosis.

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LYMPHOID TISSUE AND ITS RELATION TO SO-CALLED NORMAL LYMPHOID FOCI AND TO LYMPHOMATOSIS

IV. Simplification of Methods for Quantitative Analyses and Its Application to the Turkey

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Previous studies on lymphoid tissue found in parenchymal organs of chickens and ducks (Lucas, 10; Lucas and Oakberg, 11; and Lucas and Breitmayer, 9) led to the conclusion that such lymphoid areas should be considered abnormal and part of a reacting mechanism to a stimulating agent or agents. Analyses of quantitative data for different groups of chickens and for ducks revealed that the percentage of lymphoid tissue per section of pancreas in birds whose gross appearance was normal was significantly correlated with a predisposition toward the development of gross lymphoid tumors. In avian species thus far studied, the size and number of individual lymphoid areas and the percentage and total quantity of lymphoid tissue per section fell into "J"-shaped frequency distribution curves. Because these data are distributed into highly skewed curves, it was necessary that they be treated in some suitable manner which would convert the original scale of measurements into values which are approximately normally distributed. Transformations that can be used on this type of data have been discussed by Bartlett (2).

As new data have been added and comparisons made with the old, the type of probit transformation employed in the second and third papers of the series became so cumbersome and unwieldy that it was

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necessary to transfer the data to punch cards.[4] It became obvious also that certain disadvantages existed in the probit transformation as it has been used in the earlier work. If quantitative studies on the lymphoid tissue are to have practical value in breeding programs and in the selection of stock toward freedom from gross and clinical manifestations of lymphomatosis, and in testing of therapeutic agents, it is necessary that steps be taken toward simplification of procedure.

It is the purpose of this paper to present and evaluate the methods previously used and to study several modifications and new approaches directed toward simplification. It is the purpose, also, to apply the better methods in comparisons of lymphoid tissue in the pancreas of turkeys to that found in other species and groups of birds previously studied (Lucas and Breitmayer, 9; and Lucas and Oakberg, 11).

Materials and Methods

The pancreases from 60 turkeys were obtained at an eviscerating plant.[5] The first 5 were Bronze variety of turkeys and the remaining 55 were White Hollands and were from one farm. Of the White Hollands, 9 were males and ranged in weight from 17 to 23.5 pounds. The remaining 46 were females ranging from 7 to 15.5 pounds. Records on the ovary indicated that 38 were in production, or had been recently, and the ovaries of 7 were inactive. The ovary was not seen in 1 case. There were no gross pathological alterations suggestive of lymphomatosis in any of the birds.

The ducks and chickens were those reported in previous publications of this series. These were used for comparison with turkeys and to test results from newer techniques of analyses with those previously used.

One or more cross sections of the pancreas were taken as sample tissues from each bird. Sections were cut 5 microns thick. Enlarging and tracing the sections and their contained lymphoid areas were done with a Bausch and Lomb microprojector.

The sections of the pancreas were traced, using a 32 mm. micro

[4]We are grateful to Mr. Francis B. Martin and his staff in the Tabulation Department of Michigan State College for their continued interest and help during the past year in setting up and operating the punch card machines. Without this help, the results obtained might well have been indefinitely delayed.

[5]We are indebted to Mr. A. G. Lohman of the Hamilton Farm Bureau, Hamilton, Michigan, who kindly reorganized the processing procedure so that we might obtain tissues and data which we desired for this study.

Tessar objective without tube or eyepiece, at a distance of 18.5 inches between stage and an $8\frac{1}{2} \times 11$ inch sheet of paper used for tracing the projected image. This gave a magnification of about 12.5 times. The projecting distance was adjusted so that 1 mm. on the stage micrometer equalled 0.5 inch on the paper. Thus a section on the slide as large as 21×16 mm. or 336 sq. mm. could be traced on one sheet. The average cross section size for the chicken pancreas is 25 sq. mm., for the domestic duck about 100 sq. mm., and for the turkey about 50 sq. mm.

After the sections were outlined, the distance was readjusted so that 1 mm. projected through a 16 mm. objective, a tube and a $5\times$ eyepiece equalled 3 inches. The same projection distance was used for the 8 and 4 mm. objectives as for the 16 mm.

The section was scanned completely with the mechanical stage and in so doing any lymphoid areas observed were traced. An objective was selected which would give a projected image larger than about a quarter of a square inch and yet sufficiently small so that 6 to 12 areas could be traced on the same page.

The outline drawing of the whole section and the individual lymphoid areas were traced with a compensating polar planimeter. Two measurements were made of each figure, both with the ball and socket joint to the left. The ball and socket was used only on one side because other inherent sources of error did not justify the increased time and labor involved in obtaining somewhat increased accuracy of measurement by using the joint on opposite sides.

A factor was derived for converting planimeter readings to actual area on the slide. Obviously there would be a different conversion factor for each magnification used. Up to the present, the time required for all steps in obtaining basic data has been much less than that required for the statistical calculations involved in the analyses but it is hoped that the suggestions made here will set up a standard procedure and reduce time and labor to a more reasonable level.

Results from Methods Tested

Use of Probits with Frequencies of Variables Combined

The use of probits based on the cumulative percentage of the combined frequencies of the variables involved was suggested to Lucas and Oakberg (11) by Dr. Jay L. Lush. The use of probits is dis-

cussed in various statistical texts but not very frequently in elementary texts. For this reason, and also to provide a point of discussion and suggestion for modification, the steps involved in this procedure have been set up in Table 1. Combined frequencies are given in column 5.

Only half the turkey section was used to compare with the whole section from the chicken. This was done because, on the basis of information available thus far (Lucas and Breitmayer, 9), it had been shown that an analysis is possible when the ranges and means for section areas of the two groups being compared are approximately equal. The range in section size for the turkeys was 24.58 to 88.45 sq. mm. with a mean of 47.27 sq. mm. of which half is reasonably close to an average of 23.1 sq. mm. for all Laboratory chickens and 25.2 sq. mm. for Farm chickens. The lymphoid areas within sections were distributed by means of a table of random numbers to each half section. This treatment (described below) is applicable only for data of this type where the arithmetic values for section size are approximately normally distributed and the lymphoid areas are not. The amount of lymphoid tissue is a function of section size but measurements of lymphoid tissue must undergo a logarithmic or some similar transformation in order to give an approximately normal distribution. In other words:

$$w = \text{lym.} \times \text{S.A.} \tag{1}$$

When w is the product of the quantity of lymphoid tissue (lym.) times the sample area (S.A.) but lym. is logarithmically distributed and applied to the equation gives (2) but now log w is not normally distributed

$$\log w = \log \text{lym.} + \log \text{S.A.} \tag{2}$$

because log S.A. is not normally distributed. But if S.A. is held constant then log w = log lym. In other words, keeping the sample size comparable for all groups of birds or tissues within birds, the factor of section area can be ignored and removed from the equation. A reduction of the larger mean for a group of birds to equal the smaller mean seemed the simplest adjustment to make. It is possible to reduce a large sample to a smaller one and by a table of random numbers distribute the lymphoid areas among the fractions but there is no known way to estimate the amount of lymphoid tissue which would be present if a small sample were expanded.

TABLE 1

SAMPLE SHOWING NORMALIZATION USING A PROBIT TRANSFORMATION WITH FREQUENCIES COMBINED, PERCENTAGE OF LYMPHOID TISSUE, TURKEYS* VS. LABORATORY RESISTANT CHICKENS

1	2	3	4	5	6	7	8	9	10	Coded value 11	Coded value 12
Class range	Class mean	Frequency for turkeys	Frequency for Lab. resistant chickens	Combined frequency for two groups	Area of class	Cumulative areas	Cumulative —0.5	Ordinates	Diff. between consecutive ordinates	Mean of class	Adj. values if 1st class is minus
.00	.00	22	86	108	.5094	.5094	.009	.3988	.3988	—.783	.000
.00001- .09999	.05	16	27	43	.2028	.7122	.212	.3412	.0576	+.284	1.067
.10 - .19	.15	10	14	24	.1132	.8254	.325	.2578	.0834	.737	1.520
.20 - .29	.25	3	7	10	.0472	.8726	.373	.2081	.0497	1.054	1.837
.30 - .39	.35	3	2	5	.0236	.8962	.396	.1806	.0275	1.166	1.949
.40 - .49	.45	2	3	5	.0236	.9198	.420	.1487	.0319	1.353	2.136
.50 - .59	.55	2	1	3	.0141	.9339	.434	.1283	.0204	1.442	2.225
.80 - .89	.85	1	1	2	.0094	.9433	.443	.1144	.0139	1.474	2.257
.90 - .99	.95		1	1	.0047	.9480	.448	.1064	.0080	1.695	2.478
1.10 - 1.19	1.15	1	3	4	.0189	.9669	.467	.0736	.0328	1.738	2.521
1.30 - 1.39	1.35		1	1	.0047	.9716	.472	.0643	.0093	1.970	2.753
1.40 - 1.49	1.45		1	1	.0047	.9764	.476	.0565	.0078	1.653	2.436
1.60 - 1.69	1.65		1	1	.0047	.9811	.481	.0464	.0101	2.140	2.923
1.80 - 1.89	1.85		1	1	.0047	.9858	.486	.0357	.0107	2.267	3.050
2.10 - 2.19	2.15		1	1	.0047	.9905	.491	.0243	.0114	2.415	3.198
2.90 - 2.99	2.95		1	1	.0047	.9953	.495	.0145	.0098	2.076	2.859
88.30 -88.39	88.35		1	1	.0047	1.0000	.500	.0000	.0145	3.072	3.855
	Total	60	152	212							

Col. 6: $\frac{\text{No. for class}}{212}$

Col. 9: From Table 12 (Holzinger, '31)

Col. 11: $\frac{\text{Values in Col. 10}}{\text{Values in Col. 6}}$

*Data for turkeys are for a half section.

In analysis of variance, using coded values as set up in Table 1, size of individual lymphoid areas, percentage of lymphoid tissue and number of areas gave no significant difference between the two halves. One of the half sections was chosen arbitrarily to be used in analyses with chickens.

The sign is determined by the ordinate value. All those up to and including the maximum value for the ordinate are negative and all those beyond are positive. In column 12 (Table 1) a +.783 has been added to each figure obtained in column 11. Thus negative values are eliminated from the analyses. The chief objection to this method of analysis is the fact that a new set of coded values must be worked out for each combination studied.

Use of Probits with Frequencies of Variables Separated

As already mentioned use of combined frequencies is time consuming because the SX and SX^2 obtained as part of one analysis cannot be used as part of another analysis. Therefore, the turkey data, in relation to those of ducks and Farm and Laboratory chickens, were arranged so that normalized values based on the use of probits were calculated for the classes within groups of data, such as a set of values for the ducks, another for the turkeys, another for resistant Laboratory chickens, and so on. Thus coded values were worked out for data in column 3 and also for data in column 4.

It was determined to what extent different results were obtained when analyses were made using "combined" and "separate" frequencies for pairs of groups of variants. A comparison of 17 pairs of variance analyses gave the same level of significance in 12 cases, a lower level of significance when separate frequencies were used in 2 cases and a higher level of significance in 3 cases. The shift in the F value was actually never very great. Certainly the two methods do not always give the same results.

In combined frequencies the assumption is made that the samples are taken from a common population, and the analyses were made to test the correctness of the assumption. When used as separate frequencies it is not assumed that the samples are drawn from a common population. We do not have the information at present to determine which is the more critical test.

For this type of data, probits do not seem too satisfactory but the most serious objection is that they take no account of the actual

values of the class means which went into the curve as shown in Table 1. The shape of the curve is determined by the number and sequences of classes and not their real values. The last class having a mean of 88.35 would be the same on a probit scale if it were only 3.05. This difficulty is corrected by the use of a logarithmic transformation so that weight is given to the actual values.

Use of Logarithmic Transformation

After the previous papers were published (Lucas, *et al.*, 9, 11) it became apparent that sizes of individual lymphoid areas are approxi-

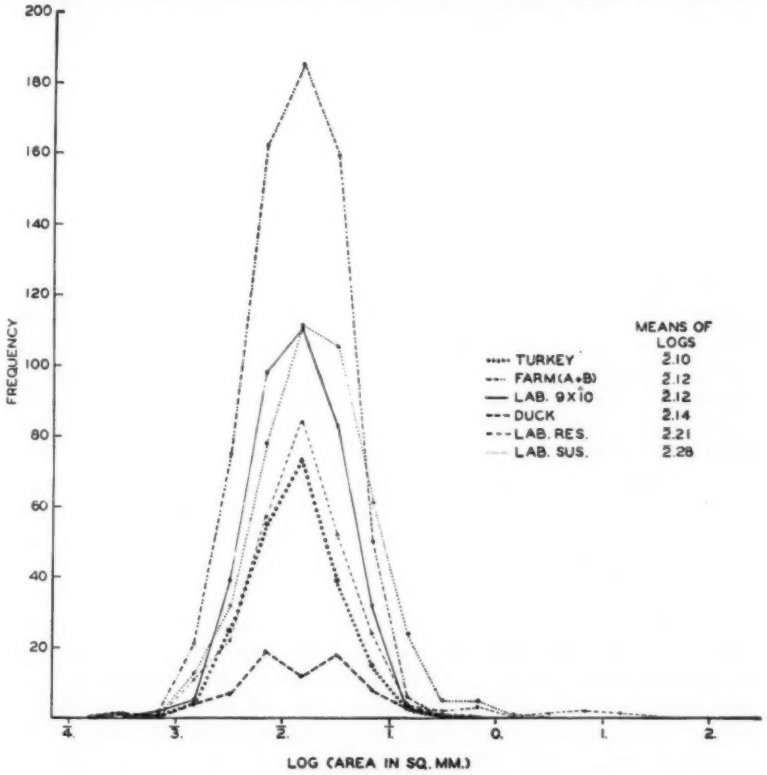

FIGURE 1

A comparison of sizes of individual lymphoid areas for turkeys, chickens and ducks, using the logs of the areas.

mately normally distributed when a logarithmic transformation is applied. When plotted graphically the similarity of their distribution and their means is shown in Figure 1. A comparison of means obtained by variance analyses is given in Tables 2 and 3.

TABLE 2
RESULTS OF VARIANCE ANALYSES BETWEEN BIRDS FOR SIZE OF INDIVIDUAL LYMPHOID AREAS BASED ON LOGARITHMIC TRANSFORMATION × 10,000

No. of individuals with lymphoid areas	No. of lymphoid areas	Groups	Between lines (for Laboratory chickens)	Between birds
38	73	Ducks	—	N.S.
66	263	Laboratory resistant chickens	N.S.	N.S.
54	216	Turkeys	—	*
41	372	Laboratory 9×10	—	*
84	660	Farm chickens	—	**
86	438	Laboratory susceptible chickens	N.S.	**

N.S. = Not significant.
* = Significant at 5 per cent level.
** = Significant at 1 per cent level.
— = No data by lines for these groups.

The groups showed different levels of variance in the size of individual lymphoid tissues between individuals. The ducks and Laboratory resistant chickens showed the highest degree of uniformity. The turkeys and Laboratory hybrid groups are next and the Farm and Laboratory susceptible chickens show the greatest variability. The result given in Table 2 for the Laboratory resistant chickens was obtained only after the bird showing the definite neoplastic condition had been removed from the calculation. Its removal seemed justified since histologically it was different from all the other cases. When it was included there were significant differences between lines and between birds. There was more lymphoid tissue in this one section than the total lymphoid tissue in all the other sections for the resistant group of chickens.

A comparison of means by the use of a variance analysis, after the variance within and between birds had been used as the error term, is summarized in Table 3. The groups have been arranged in order of increasing means of the logarithms. The ranking has no significance except in the placing of the Laboratory susceptible group. The lymphoid areas here are significantly larger than those found in turkeys, Farm chickens and the hybrid group of Laboratory chickens

TABLE 3

RESULTS OF VARIANCE ANALYSES FOR SIZE OF INDIVIDUAL LYMPHOID AREAS BASED ON LOGARITHMIC TRANSFORMATION × 10,000

	Turkeys	Farm chickens	Lab. 9×10 chickens	Ducks	Lab. resist. chickens
Turkeys					
Farm chickens	$\bar{2}.095$ N.S. $\bar{2}.116$				
Lab. 9×10 chickens	N.S. $\bar{2}.125$	$\bar{2}.116$ N.S.			
Ducks	N.S. $\bar{2}.143$	N.S.	$\bar{2}.125$ N.S.		
Lab. resist. chickens	N.S. $\bar{2}.213$	N.S.	N.S.	$\bar{2}.143$ N.S.	
Lab. susc. chickens	*** $\bar{3}.283$	***	***	N.S.	$\bar{2}.213$ N.S.

N.S. = Not significant.
*** = Significant beyond 0.1% level.

but are not significantly larger than those of ducks and Laboratory resistant chickens.

It should be recognized that the use of size of individual lymphoid areas is not a reliable means of measurement for the whole group. Comparisons are based only on those which have lymphoid areas, and whether there is a large or small proportion of the population showing no lymphoid tissue in the section does not enter into these analyses. It merely tells us that in respect to size of their respective lymphoid areas there is little, if any, real differences between the groups, with the exception of the Laboratory susceptible chickens.

Application of Logarithmic Transformations to Groups of Data Having a Zero Class

The advantages of logarithmic transformation over the use of probits in analyses of size of individual lymphoid areas led us to apply this method to types of data where a zero class existed. A preliminary examination of percentage of lymphoid tissues (Fig. 2) indicated that this variant as well as size was distributed approximately logarithmically. The section size for each species of bird has been adjusted to give similar means. In Figure 2 the zero class has been listed at the

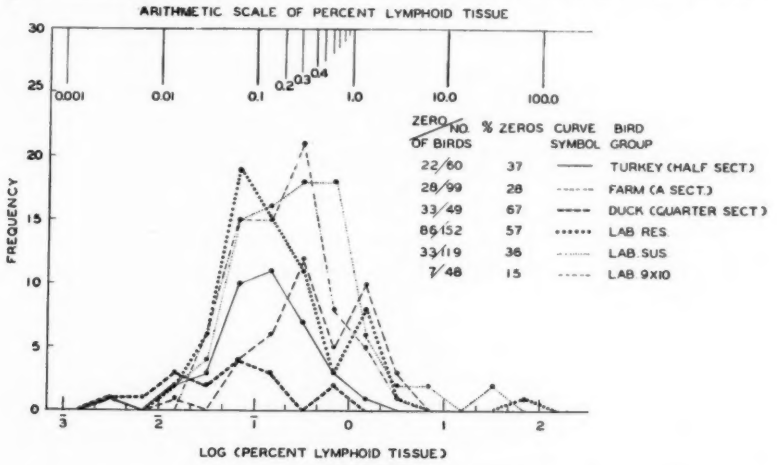

FIGURE 2

A comparison of percentages of lymphoid tissue for turkeys, chickens and ducks. A logarithmic scale is used below and an arithmetic scale above.

side of the curves. The size of the zero class varied greatly for different groups. At the top of Figure 2 has been indicated the units of the arithmetic scale which were used in the second and third papers of this series. It is observed that the class unit of 0.1 per cent previously used is relatively close to the mean of the logarithmic values. To test the validity of a logarithmic transformation for turkey half section, the zero class was combined with the next higher class and the logarithm of the upper class limit of each arithmetic class was plotted against the probits of the cumulative frequencies for the classes (Table 4 and Figure 3). For this test a class unit of 0.01 sq.

TABLE 4
TURKEY (HALF SECTON). PERCENTAGE OF LYMPHOID TISSUES. CONVERSION OF ARITHMETIC CLASSES TO LOGARITHMS AND PROFITS.

Class range	Log 10 × of upper class limit	Frequency	Cumulative percentage of frequencies	Probit*
.000- .100	.000	38	63.33	5.3406
.101- .200	.301	10	80.00	5.8416
.201- .300	.477	3	85.00	6.0364
.301- .400	.602	3	90.00	6.2816
.401- .500	.699	2	93.33	6.5009
.501- .600	.778	2	96.67	6.8388
.701- .800	.903	1	98.33	7.1272
1.101-1.200	1.079	1	100.00	—
		60		

*Taken from Table IX, Fisher and Yates (5).

mm. was used. Since the points fall rather close to a straight line, it is concluded that the logarithmic transformation is useful. When employed in analyses the logarithm of the mean of the class was used instead of the upper limit, since it is the means of the separate classes which determine the shape of the curve.

Percentages of lymphoid tissue are useful for comparisons but contain an inherent error in that larger sections will appear to have larger amounts than smaller samples. This was brought out in the second and third papers (11, 9). Oakberg and Lucas (12) used a covariance analysis to correct for section size. Such a procedure has advantages over the use of percentage values but one experiences the same difficulties found in the use of probits if correction is not made in this type of data to give similar section size means for the groups compared. Class means of 0.01, 0.03, 0.05 sq. mm., etc., were used in assembling the frequency data for total lymphoid tissue. The zero

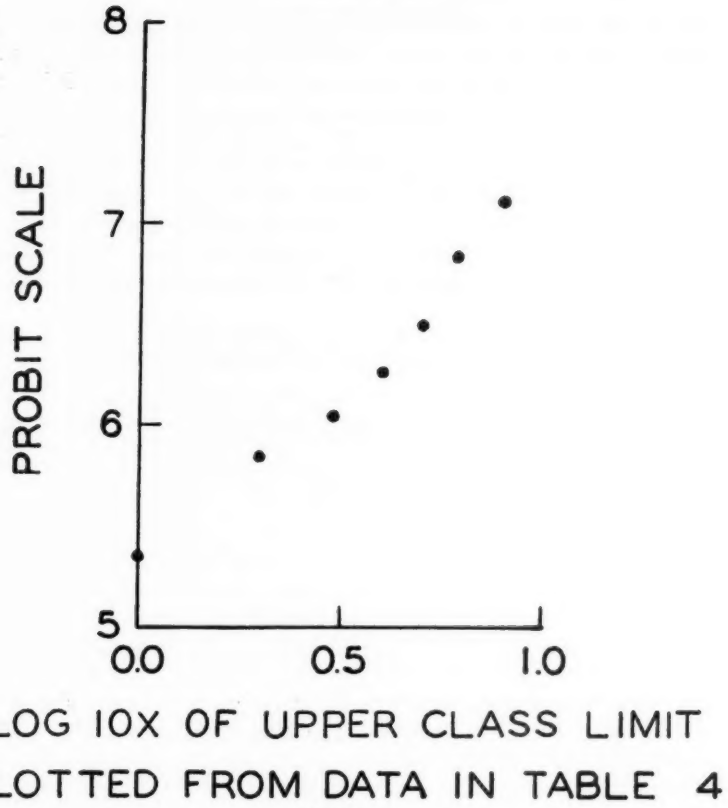

FIGURE 3
A test for the normality of distribution of data.

class was included with the 0.01 sq. mm. group and the normalized value employed in the analysis was the logarithm of the class mean times 100. The mean for the respective groups given in the corners of blocks in Table 5 are normalized values and not actual values. The size of the section used in the analysis influences the value of the normalized means. The small unit has the lower mean; for example, a duck quarter section has a mean of 0.085 and a half section a value of 0.213.

Results of the covariance analysis are presented in Table 5. Comparisons were made between two groups at a time. In the analyses X is the total amount of lymphoid tissue (for Table 6 it was the number of lymphoid areas) and Y is the section area in sq. mm. The effects of regression of section size were subtracted from S_{x^2}. The group of chickens labeled Laboratory resistant represents the lines which had less than 50 per cent mortality associated with gross lymphoid tumors according to Method II (Lucas and Oakberg, 11); Laboratory susceptible are those lines having more than this amount of mortality associated with gross lymphoid tumors. The hybrid line, 9×10, has been removed from the inbred lines and treated as a separate group. The ranking of the various groups puts the ducks at the top with the least amount of lymphoid tissue, followed by the Laboratory resistant group, then the turkeys, followed by the Farm chickens. The Laboratory susceptible group has a large amount of lymphoid tissue and the hybrid line the most of all. The cross, 9×10, was obtained as part of the breeding program from a male of a susceptible line and females of a resistant line. The incidence of gross lymphoid tumors was low but the amount of lymphoid tissue was exceedingly high compared with that of the others. It would have been interesting to know what the incidence of gross tumors would have been in the F_2 generation when the possible effects of heterosis had diminished. It is planned to study further the effect of hybridization on the amount of lymphoid tissue.

Adjacent ranking groups, such as turkeys and Laboratory resistant chickens, turkeys and Farm chickens, and Farm and Laboratory susceptible chickens, are not significantly different from each other, but the ducks have significantly less lymphoid tissue than the nearest ranked group and the hybrid group significantly exceeds the Laboratory susceptible chickens. The results obtained by the use of a logarithmic transformation and total lymphoid itssue are essentially the same as those obtained by the use of probits, combined frequencies and percentages which were given in the previous studies and with the added advantage of a great saving in time. The number of lymphoid areas per section was subjected to the same statistical treatment as the total lymphoid tissue per section. Theoretically, if there is no significant difference in size of individual lymphoid areas for groups of birds, then the differences should depend almost entirely on number and the evidence presented earlier indicated that the individual

TABLE 5

RESULTS OF COVARIANCE ANALYSES OF TOTAL LYMPHOID TISSUE AGAINST SECTION SIZE AFTER ADJUSTMENTS TO MAKE THE MEAN SECTION SIZE EQUAL FOR THE DIFFERENT GROUPS

Rank	Ducks	Lab. resist. chickens	Turkeys	Farm chickens	Lab. susc. chickens
1. Ducks					
	.085				
	**				
2. Lab. resist. chickens	.277				
	.213	.277			
	***	N.S.			
3. Turkeys	.586	.313			
	.213	.277	.586		
	***	*	N.S.		
4. Farm chickens	.725	.501	.725		
	.085	.277	.313	.501	
	***	***	**	N.S.	
5. Lab. susc. chickens	.561	.561	.561	.561	
	.085	.277	.313	.501	.561
	***	***	***	***	**
6. Lab. 9×10 chickens	.849	.849	.849	.849	.849

N.S. = Not significant.
* = Significant at the 5% level.
** = Significant at the 1% level.
*** = Significant at the 0.1% level.

TABLE 6

RESULTS OF COVARIANCE ANALYSES OF NUMBER OF LYMPHOID AREAS AGAINST SECTION SIZE AFTER ADJUSTMENTS TO MAKE THE MEAN SECTION SIZE EQUAL FOR THE DIFFERENT GROUPS

Rank	Ducks	Lab. resist. chickens	Turkeys	Farm chickens	Lab. susc. chickens
1. Ducks					
	.748 ***				
2. Lab. resist. chickens	.949				
	.817 ***	.949 N.S.			
3. Turkeys	1.337	1.035			
	.817 ***	.949 ***	1.337 N.S.		
4. Farm chickens	1.464	1.223	1.464		
	.748 ***	.949 ***	1.035 *	1.223 N.S.	
5. Lab. susc. chickens	1.218	1.218	1.218	1.218	
	.748 ***	.949 ***	1.035 ***	1.223 ***	1.218 ***
6. Lab. 9×10 chickens	1.594	1.594	1.594	1.594	1.594

N.S. = Not significant.
* = Significant at the 5% level.
*** = Significant at the 0.1% level.

lymphoid areas had similar log means. The only exception was the Laboratory susceptible group and this was not great enough to produce any great dissimilarity between results in Tables 5 and 6. This conclusion has already been reached by Oakberg and Lucas (12) in a study of differences in amount of lymphoid tissue with age. In the present study, however, the Laboratory susceptible group showed significantly larger lymphoid areas than some of the other groups and it was of interest to determine to what extent this would change the ranking of the groups and their interrelationships (Table 6).

The class means used for numbers were 0.5, 2, 3, 4, 5, etc. The first class is more inclusive than the succeeding ones and combines the "zero" and "one" classes. This is partly the result of the discontinuities inherent in dealing with number of lymphoid areas. Theoretically it should not bias the analysis. Logarithms of class means were used and the means for the groups given in the blocks of Table 6 are logarithmic means times 10.

The results obtained from analyses based on number (Table 6) are essentially the same as for total lymphoid tissue (Table 5) which takes into account the variable size. When number was used the level of significance was increased from the 1.0 to the 0.1 per cent level of significance in two cases, from the 5 to the 0.1 per cent level in one case and decreased from the 5 to the 1 per cent level in one case. In no instance was there a shift from a significant to a non-significant level, and more important is the fact that order of ranking remains identically the same for the two sets of analyses.

The use of numbers alone is adequate for comparisons of populations but may not be sufficiently exact for comparisons between individual birds, since sometimes there were found to be significant differences in size of lymphoid areas between birds as has been brought out in Table 2. The fact that the number of lymphoid areas gives essentially the same result as total lymphoid tissue permits a great saving in time because this eliminates the necessity of projecting, tracing, and measuring the areas of the individual lymphoid foci. All that is needed are the area of the section and a count made under the microscope of the number of lymphoid areas; whether these areas are large or small can be ignored when groups of birds are compared. This is applicable, of course, only when the log means of size of lymphoid areas are comparable for the two groups being compared.

Histological Observations and Discussions

The lymphoid lesions in turkeys were no different from those found and described in chickens and ducks. There were the same destruction of adjacent acinar tissue, lymphocytic infiltration between acini, focal encapsulated areas which are thought to arise from plugged blood vessels, and destruction of islets of Langerhans and intrinsic ganglia. The photographs presented for chickens (10) and ducks (9) could just as accurately illustrate the conditions found in turkeys. All of these lymphoid areas, including the smallest, are considered abnormal. It should be emphasized that these lymphoid reactions are observed in birds which, with the exception of 14 of 418 chickens, showed no gross lesions characteristic of clinical lymphomatosis and no such lesions were noted in either ducks or turkeys. No gross lesions were observed in the pancreas in any case examined. In only one of the 522 birds examined thus far was the microscopic picture such that it could be classified definitely as a neoplasm. There were large lymphoid cells with vesicular nuclei, large nucleoli and numerous abnormal mitoses. This one case was not sufficient to bias the analyses, and thus for all practical purposes the significant correlation between the amount of lymphoid tissue and the incidence of gross lymphoid tumors is based on lymphoid tissue which is abnormal but shows only two criteria of neoplasia, namely, invasion and destruction of adjacent tissue. Numerous earlier investigators observed lymphoid areas in a wide variety of avian species and organs. Their various opinions have been reviewed (10, 11, 12). No one, however, has previously established the point that the amount of lymphoid tissue is a measure of the predisposition toward the development of gross lymphoid tumors.

The lymphoid reactions before they reach a definitely neoplastic condition are often associated with blood vessels (Lucas, 10) and by their distribution suggest a systemic reaction involving primarily the vascular bed and adjacent adventitial tissues. This suggestion implies that the agent or agents causing gross tumors are present in these reaction foci. This does not imply, however, that the extent of lymphoid involvement is a measure of the amount of agent present; all the intrinsic and extrinsic interactions of a host-pathogen relationship enter into the final visible reaction. Neither does it imply that only a specific agent or group of agents can stimulate the development of

lymphoid foci, although thus far aspergillosis in ducks (Lucas and Breitmayer, 9) did not increase the amount of lymphoid tissue in the pancreas. More information is needed along these lines.

The lymphoid reactions may be entirely non-specific and an increased quantity of stimulated lymphoid tissue, from whatever cause, may merely increase the statistical probability that a cell will become definitely neoplastic. Such a postulate, however, makes it rather difficult to account for the filtrable agent often recovered from neoplastic lymphoid tumors, unless the filtrable agent is a product of the tumor rather than its cause. There is no experimental evidence, thus far, that such is the case.

On the basis of the hypothesis suggested in this and previous papers, it would seem that the ranking of the different species and groups of birds, as shown in Tables 5 and 6, fairly well evaluates the predisposition toward the clinical manifestation of lymphomatosis. There might be some question in respect to placement of turkeys in relation to Laboratory resistant chickens, namely, that the clinical reports on the incidence of lymphoid tumors in turkeys are relatively few (Durant, 4; Andrews and Glover, 1; Downham, 3; Harnden and Smith, 6; and Hoffman, 7) and although turkeys are affected, the indications are that the incidence is lower than the average for chickens. On the basis of such reports it might more logically be ranked between the ducks and Laboratory resistant chickens. The results may be influenced by the fact that 55 of the 60 turkeys came from one farm. This factor of selection with regard to turkeys is in contrast to that of farm chickens, which came from ten different farms.

Summary

1. Evaluation has been made of the application of probit transformations useful in quantitative analysis of lymphoid tissues.

2. Variations in size of individual lymphoid areas are distributed approximately normally when a logarithmic transformation is applied.

3. Percentage of lymphoid tissue, total lymphoid tissue and number of lymphoid areas per section are distributed approximately logarithmically also.

4. A method has been developed whereby a zero class can be included in logarithmic transformation.

5. It was found that analyses based on number of lymphoid areas

gave similar results to those for total lymphoid tissue. This effects a considerable saving in time with little, if any, loss in accuracy.

6. The results of analyses indicated that in respect to quantity of lymphoid tissue the groups ranked in the following order: ducks, Laboratory resistant chickens, turkeys, Farm chickens, Laboratory susceptible chickens and, finally, a Laboratory hybrid.

7. Histologically the lymphoid areas in the pancreas of turkeys show the same evidences for abnormality that were found in species previously studied. The results in relation to lymphomatosis are discussed.

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SOME ASPECTS OF THE ROLE OF HORMONAL AND NUTRITIONAL FACTORS IN SKELETAL GROWTH AND DEVELOPMENT*

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In the study of skeletal growth and development various methods are being employed: Body weight has been used as an indicator of the adequacy or inadequacy of skeletal growth, although determinations of bone length are more accurate in this respect. X-ray observations permit an estimate of the degree and progress of calcification and ossification of the bones, that is of skeletal development; qualitative and quantitative biochemical investigations have advanced the knowledge of the basic materials needed for the building up of the normal skeleton and essential for physiological growth. However, none of these methods allows a close insight into the mechanisms involved in skeletal growth and development which represent a complex of multiphasic processes.

Developing from undifferentiated connective tissue, in its final structure the bony skeleton is histologically, biochemically and biophysically different from the original material, and in addition a third type of tissue, again of different character—cartilage—may appear as an intermediary product. A study of the mechanisms involved in skeletal growth has, therefore, to take into account the histologic make-up of the tissues, that is their cells and matrix. The significance of the presence or absence of certain biochemical substances may be appraised correctly only if their correlation to or their localization in certain sites can be established. In this respect, microscopic examination of the bones seems to be the most satisfactory method for observations of the life cycle of the various skeletal cells and of

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factors that influence this cycle. This point of view stressed by us for many years, has more recently been adopted also by investigators who had heretofore used weights and measurements as criteria of skeletal growth. Histogenetic studies of the mechanisms underlying endochondral ossification may contribute also in a general way to the understanding of the mutual relationship between cells and intercellular matrix and of the relationship between the progressive changes of growth and the regressive changes of ageing. The knowledge into these mechanisms is at the present time limited, and little is known about the biochemical reactions associated with these processes. It is to be expected, however, that through the application of cytochemical and cytophysical techniques more information will be forthcoming than was obtained in the past by means of histologic analysis alone.

It is the purpose of this paper to review briefly some results of our microscopic investigations on growth, development and ageing of cartilage and bone and on experimental modifications of these processes.

The Skeletal Time Curve

In mammals, growth of the long bones is contingent upon the presence at both ends of the bone of a "patent" epiphyseal disk. This growth zone is represented by a plate of cartilage cells arranged in vertical columns and developing from an undifferentiated layer of mesenchymal cells adjoining the epiphyses. As long as growth in length proceeds, each epiphyseal cartilage cell passes through a successive cycle of proliferation, hypertrophy, senescence and death. The peak of hypertrophy coincides with the maximum development of a cartilage cell. Once this stage has been reached, the cell undergoes age changes, in particular calcification and subsequent destruction owing to erosion by metaphyseal cells or capillaries. Whereas the cartilage cells are broken down, spurs of calcified matrix remain preserved and form a solid base for the deposition of connective tissue cells which round off and develop into osteoblasts. The latter likewise proliferate, enlarge and lay down organic bony matrix. Finally mature bony trabeculae containing osteocytes are being formed. In due course of time these spicules are resorbed, giving way to the expanding diaphyseal marrow cavity. As long as growth and development of cartilage proceed, growth in length goes on at a corresponding rate. With advancing age of the individual, proliferation of the cartilage cells slows down, whereas regressive changes as well as resorp-

tion of cartilage by constituents of the metaphyseal tissue increase in intensity. Thus a reduction of the height of the vertical cartilage cell rows, a decrease in the number of cartilage cells, and a narrowing of the epiphyseal disks result, while at the same time larger amounts of hyaline intercellular substance appear between the individual cartilage cell columns. Finally, the epiphyseal disks are converted into a narrow layer of small inert hyaline cartilage in which the cells are separated from each other by varying amounts of dense calcified matrix and delimited from the metaphysis by a bony plate. In many higher species the resorption of this inert or ossified cartilage as well as of the subepiphyseal bony lamella proceed still further leading to epiphyseodiaphyseal union, and at best scanty remnants of the previous growth zone may persist throughout life. In other species such as rat and mouse, a narrow layer of the inert epiphyseal cartilage or parts of such plates may be preserved into old age showing communications of varying size between epiphysis and metaphysis. Simultaneously with the ageing processes in the cartilage, the bony structures change from long slender spicules into short, coarse and interlacing trabeculae. Owing to continued resorption also the bony metaphyseal spicules are eventually dissolved.

These processes constitute parts of the skeletal time curve. While the fundamental processes of epiphyseal growth and development are essentially the same in different species and in different strains within a given species, there are differences as to the time of onset and the duration of the individual phases of growth, regression and resorption. Within individual strains, however, the skeletal time curve is remarkably constant, and there is a good correlation between the rate of ageing of the skeletal tissues and that of other tissues as pointed out by Loeb (1940). It has thus to be assumed that the skeletal time curve is at least partly genetically determined. Of the strains examined, strains C57 black and CBA show a slow, strains C3H and dba a fast rate of skeletal growth and development. In addition to these strain differences, the sex of the individual influences the progress of the skeletal time curve. In human beings as well as in animals, the female skeleton develops more rapidly than that of the male. This difference becomes particularly striking about the onset of sexual maturity. Later in life, skeletal development and ageing of the male is accelerated over that in the female, and the bones of the males may then appear older than those of females of corresponding chronological age.

Breeding females in turn show a more rapid progress of skeletal growth and ageing than virgin females. These observations indicate that hormones may influence the course of the skeletal time curve. By means of withdrawal of intrinsic or by administration of exogenous hormones, some of these hormonal effects could be demonstrated experimentally (Silberberg and Silberberg, 1943, 1949).

The Effects of Hormones on Skeletal Development

Investigations on the growth-promoting and growth-inhibiting effects of purified fractions of anterior hypophysis have been carried out particularly in hypophysectomized animals. The role of these hormones as far as growth of cartilage is concerned has been reviewed repeatedly (Evans, 1941; Gardner and Pfeiffer, 1943; McLean, 1943; Long, 1942, 1948, and others).

While studying the effects of prolonged administration of various hormones in animals with intact hypophyses, we noted that their effects on growth are invariably accompanied by influences on the processes of ageing; Crude anterior hypophyseal extracts as well as Antuitrin G cause softening of the cartilaginous matrix and stimulate phase I of the skeletal time curve (proliferation and hypertrophy of cartilage). However, these substances intensify at the same time phase II (regressive changes of cartilage and ossification) and also phase III (resorption of cartilage and bone). As a consequence, premature closure of the growth zones and premature epiphyseo-diaphyseal union ensue. Thus in spite of the initial growth spurt gigantism was not produced.

Thyroid hormone likewise accelerates phase I, but this hormone stimulates hypertrophy of cartilage more than proliferation; moreover, prolonged administration leads to condensation of the cartilaginous matrix and hastens the onset and progress of phase II (regressive changes) and phase III (resorption) to such an extent that the latter processes rapidly outbalance those of growth (phase I). Thus epiphyseo-diaphyseal union occurs before the growth capacity of the cartilage has been exhausted, and stunting results. In thyroidectomized animals, on the other hand, growth and development of cartilage and resorption of bone are slowed down; under these conditions stunting results from retarded development; however, more pronounced than the effects of thyroidectomy on growth are the retardation of epiphyseal closure and epiphyseo-diaphyseal union.

Parathyroid hormone in the presence of adequate amounts of calcium advances phases II and III (skeletal ageing) without stimulating phase I (growth of cartilage), and may thus likewise produce dwarfism.

Estrogenic hormones inhibit phase I (growth and development of cartilage), and promote phase II (regressive changes) by increasing calcification and condensation of the matrix, processes which are favorable to bone formation. Again the inhibition of growth in conjunction with the intensification of regressive changes and of ossification may lead to stunting of the skeleton. However, the processes characteristic of phase III, resorption of cartilage and bone, are inhibited by estrogenic hormone. As a consequence, the individual bones are thicker and more solid than usual and the expansion of the marrow cavities is incomplete. Removal of the ovary as well as administration of progesterone are followed by a retardation of both regressive changes and of bone formation so that the skeleton appears delicate, and the tissues appear for a certain length of time at least younger than corresponds to their chronological age. Over prolonged periods of time, however, ovariectomy is unable to prevent the progress of age changes, and in the end resorption of cartilage and bone proceed at a rapid rate.

Androgenic hormone accelerates phase II and the onset of phase III (skeletal ageing) in particular by hastening the development of cartilage. If the processes of ageing predominate over those of growth, dwarfism results. However, in hypogenital males, the hormone stimulates growth of cartilage more than processes of ageing, and under these conditions the hormone may act as a growth-promoting factor. This growth stimulating effect of the male sex hormone also manifests itself in orchidectomized animals in which development of cartilage is retarded.

Effects of Nutritional Factors

The effects of the A, C, and D vitamins on skeletal growth have been extensively studied and reviewed (Eddy and Dalldorf, 1944; Brody, 1945; Wolbach, 1945, 1947; Elvehjem and Krehl, 1947, and others). Less is generally known, however, about the role of B-complex vitamins in skeletal growth except for the fact that in the absence of individual constituents as well as of the whole B complex growth was found to be inhibited to a varying degree. We were, therefore, interested in finding out whether this growth inhibition represented a

non-specific effect comparable to that obtained by caloric restriction or whether the individual members of the B complex specifically promote one or the other phase of skeletal growth. While the evidence is not yet complete, it seems that both specific and non-specific effects are exerted by the various members of the vitamin B complex (Silberberg and Levy, 1948).

In complete B complex deficiency as well as in pantothenic acid deficiency, proliferation and development of cartilage and bone formation cease altogether. In animals fed a pyridoxine free diet, the inhibition of growth manifests itself particularly in a retarded or suppressed development of cartilage cells, and in a deficient development of osteoblasts from connective tissue cells, a disturbance which in turn results in inadequate bone formation. Riboflavin deficiency causes an acceleration and intensification of phase II (regressive changes) in the cartilage, but this vitamin has apparently less pronounced influence on the processes of phase I (growth of cartilage) and on bone formation. The effects of the members of the vitamin B complex on skeletal growth are evident also in old mice. In these animals only the development of osteoblasts is severely affected by the vitamin deficiencies.

The Effect of Caloric Underfeeding

Underfed guinea pigs or rats (Osborne and Mendel, 1914; Jackson, 1925; Silberberg and Silberberg, 1940; McCay, Sperling and Barnes, 1943; Saxton, 1945) kept on a balanced diet restricted only in calories grow at a diminished rate and their skeleton develops more slowly than usual. Endochondral ossification as such, however, is undisturbed. In the end, smaller amounts of bone are formed than ordinarily, but the osseous substance deposited is dense, properly calcified and of regular architecture. The inherent tendency of the growth capacity of the cartilage to assert itself even under adverse conditions was strikingly demonstrated in the undernourished rats. Whereas drastic reduction of weight occurred in the soft tissues such as liver, heart, spleen, endocrines, etc., linear skeletal growth was decreased by only 8 to 10% (Saxton and Silberberg, 1947). Histologically, the effect of underfeeding consists primarily of a diminution of both the proliferation of cartilage and the formation of bone. Phase I, that of growth, is, however, prolonged as compared with the normal course of skeletal development. Phases II and III set in at a later age than

ordinarily, the intensity of degenerative and resorptive processes being decreased and their progress being delayed. The response of the skeletal tissues is thus in keeping with that of other tissues under these conditions (Saxton, 1945): Various internal organs show a retardation of all kinds of age changes, and in the endocrine glands decreased growth and development result in a "hypotypical" condition (Loeb, 1911, 1917). Even in senile rats, 900 days old and older, which had been underfed throughout their life, skeletal growth could still be resumed, and a more or less complete restoration of growth in length ensued when the animals were refed a complete diet (McCay, Sperling, and Barnes, 1943).

Enriched Diets: While during the past decade a great deal of attention has been focused on the effects of dietary deficiencies, data on the influence of enriched diets on skeletal growth and development are scanty or completely lacking. We have, therefore, more recently begun to investigate the influence of excessive amounts of certain dietary constituents on skeletal growth and development.

High Fat Diets: In mice, a diet containing 30% fat in addition to adequate amounts of protein and carbohydrates markedly hastens skeletal growth and development. In particular, hypertrophy of the cartilage (phase I) is accelerated, and more and coarser bone is laid down than usual. An intensification of phases II and III goes hand in hand with the acceleration of phase I. The regressive changes in the cartilage and resorption of cartilage and bone also proceed at an increased rate. Thus in three months old male mice of strain C57 black fed the high fat diet for two months from the time of weaning, the microscopic appearance of the growth zones is comparable to that found ordinarily at the age of six months, and in mice six months of age and receiving the high fat diet from the time of weaning the structural age of the skeleton compares with that seen ordinarily at the age of one year. Whether these changes in the skeleton are specifically due to the fat content of the diet or to the high caloric intake is still undecided and presently under investigation.

High Casein Diet: In mice of strain C57 black fed a diet containing 53% casein, growth and development of the epiphyseal cartilage (phase I) is accelerated. However, the onset and course of regressive and resorptive processes (phases II and III), is not hastened in proportion to the stimulation of growth. Thus a more youthful microscopic appearance of the skeletal tissues results due to a delay of

phases II and III. This delay in the progress of ageing is temporary only and subsequently compensated for by a more rapid course of regressive changes, so that in female mice of strain C57 black at six months of age the skeletal development of the control animals is only slightly ahead of that of the test animals.

Some General Considerations

The effects of endocrines on growth of bones and joints—in accordance with the catalytic character of hormones—manifest themselves in the speed with which certain processes of growth and ageing take place. As a rule, the age changes produced in the skeleton by extrinsic hormones do not differ qualitatively from those occurring spontaneously with advancing age and partly governed by intrinsic hormones. Only rarely does the ultimate degree of experimentally induced age changes in the skeleton exceed the most advanced degree of age changes noticeable in untreated senile animals. These hormonal effects which take place on a microchemical level inside the cells or in the intercellular matrix are reflected in the microscopic appearance of the tissues; the latter actually represent the sum total of the visible manifestations of numerous biochemical and biophysical reactions. It is a characteristic feature of the action of hormones that their effects vary with the physiological age of the target organ, in particular of the skeletal tissues. In a general way extrinsic hormones are most effective at that time of life at which also their endogenous counterparts are most active. Moreover, hormones given experimentally will intensify those processes most markedly that are already in progress at the time of the beginning of the hormonal treatment. The growth response of the epiphyseal cartilage is more pronounced in young animals than in middle-aged ones, whereas in middle-aged animals the processes of regression and resorption may be more conspicuously intensified than in young animals. Estrogenic hormone for instance is more effective in inhibiting growth in animals nearing sexual maturity than in those in which epiphyseal growth is still proceeding more actively. Ovariectomy on the other hand retards skeletal development and ageing, when performed before or about the onset of sexual maturity, but exerts hardly any skeletal effect, if carried out after the growth zones have closed.

In addition, hormonal effects on the skeleton may be modified by the species, strain, and sex of the animals used. If administered on

a body weight basis, hypophyseal hormone is most effective in guinea pigs, less in rats, and least in mice, whereas the reverse order of responsiveness is noted following treatment with estrogenic hormone. Within the same species (mouse), those strains which show a rapid rate of spontaneous skeletal development and ageing are less apt to show acceleration of the ageing processes following experimental stimulation than those strains whose skeleton naturally ages more slowly. Corresponding results are obtained if the response of the two sexes to hormonal action is compared. The young male whose skeleton physiologically ages more slowly than that of the female, reacts more actively to the administration of hormones than the latter. These strain and sex differences in response to stimulating factors are apparently not limited to those exerted by hormones. Similar differences are likewise noticeable in the reactions of slowly and rapidly ageing strains and of males and females respectively to the feeding of excessive dietary fat and protein.

The organism thus seems to possess a marked tendency to build up a skeleton that ultimately corresponds to the genetically determined pattern. In order to accomplish this, a delicate balance of nutritional and hormonal factors has to be maintained. Essential nutrients are of course a basic requirement for adequate growth, while hormones regulate processes of growth and development as well. The tendency of the skeleton to achieve its genetically determined normal structure manifests itself in a resistance to outside interference with the physiological processes of growth and development. If for any reason, whether because of nutritional deficiencies or of hormonal or other metabolic disturbances, growth is prematurely arrested, at least some of the unexhausted growth capacity is retained for a considerable length of time, and growth may be resumed upon restitution of favorable conditions. The regulating mechanisms active under these conditions are probably more complex than they appear at first sight, and involve the endocrine glands as well. Normally, the ageing processes are accelerated after the onset of sexual maturity, when large amounts of sex hormone are produced. Undernourished animals show a slowing of growth, but at the same time there is in females a suppression of ovarian activity and less estrogenic hormone is available to counteract the already reduced growth processes. Similarly, the skeletal tissues offer resistance to overgrowth: In mice and guinea pigs, the growth stimulation caused by anterior hypophyseal hormone

is of a temporary nature and is accompanied by a shortening of the growth period. This is accomplished by a sharp decline of proliferation as well as a marked and rapid increase in the rate of regressive changes. To this homeostatic mechanism may be attributed the difficulty if not the impossibility of enforcing gigantism in experimental animals.

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